REMOVAL OF TERATOGENIC 2,4-DICHLOROPHENOXYACETIC ACID USING ACTIVATED CARBON IN BIOSAND FILTERED DRINKING WATER, A CASE OF AHERO IRRIGATION SCHEME

BY

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DECLARATION

Declaration by the candidate

I declare that to the best of my knowledge, this thesis is my original work and has not been submitted for the award of any degree in any university. No part of this thesis may be produced without the prior permission of the author and/ or University of Eldoret.

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DEDICATION

I wish to dedicate this work to my departed father Jackson Kerich, to my sisters and brothers for their invaluable support, encouragement and prayers during this MSC period. Finally, to my Mama Grace Kerich, a workaholic and never tired disciplinarian who shaped my future.

ABSTRACT

Access to safe and clean drinking water is a major challenge to the people living around Ahero Irrigation (AIS). Water source in the area is constantly and increasingly polluted by agrochemical like pesticides from rice farming. Point of use treatment technology is inexpensive and effective in drinking water treatment. Bio-Sand Filter (BSF) is one of such technologies that have been implemented in Kenya and over 20 countries worldwide. While the health benefits of using a BSF in terms of reduction of diarrheal disease have been fairly well documented, little research has focused on the ability of this technology to remove other contaminants in drinking water that pose health concerns especially pesticides. 2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide extensively used in irrigation scheme. The residues of 2,4-D are present in air, water, soil and edibles. 2,4-D has been known to cause cellular mutations which can lead to cancer. The neurotoxic, immunosuppressive, cytotoxic and hepatoxic effects of 2,4-D have been well documented. But there is a lot of controversy with the teratogenic effects of 2,4-D. *Xenopus laevis* was used to evaluate the teratogenic effect of this pesticide. Structured questionnaires were administered to the people living around AIS in order to obtain information on their water sources and extent of use of 2,4-D. Then purposive sampling was used to collect water sample from key sources in the study area, then spiked with 2,4-D at different concentrations then passed through BSF, and analyzed for presence of 2,4-D using HPLC. The water that was passed through BSF was then passed through Granular activated carbon (GAC) and locally activated charcoal from bamboo (LACB) each and analyzed for the removal of 2,4-D. BSF was able to remove 2,4-D to a small amount of up to 6.1±0.43% (SE) at 5.4 mg/l initial concentrations, while GAC had the highest removal efficiency of (95.27±0.10%) (SE) and (91.49±0.15%) (SE) for bamboo at 238.7 mg/l 2,4-D initial concentration each. 2,4-D teratogenic index was 1.003. BSF is not effective in the removal of 2,4-D, hence its recommended that the water passed through BSF should be also passed through activated carbon in order to enhance safety of drinking water with respect to both the chemical and microbiological contaminants.

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LIST OF ABBREVIATIONS AND ACRONYMS

µG/l:	Microgram per liter
2,4-D:	2,4-Dichlorophenoxyacetic acid
AAPCO:	Association of American Pest Control Officials
CAS:	Chemical Abstracts Service
EC_{50}:	Effective concentration that causes malformation in 50% of test organisms
EPA:	Environmental Protection Agency
EPA:	Environmental Protection Agency
GAC:	Granular Activated Carbon
HPLC:	High Performance Liquid Chromatography
IU:	International Units
IUPAC:	International Units for Pure and Applied Chemistry
KEBS:	Kenya bureau of standards
LACB:	Locally Activated Charcoal from Bamboo
LC_{50}:	Lethal concentration that kills 50% of the population
MG/L:	Milligram per liter
NIB:	National Irrigation Scheme
NTU:	Nephelometric Turbidity Unit
pH:	$Log[H^+]$
TI:	Teratogenic index
TRF:	Tea Research Foundation of Kenya
UV:	Ultra-Violent Radiations
WHO:	World Health Organization

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CHAPTER ONE: INTRODUCTION

1.1 Background information

Water is a major component of the environment and consequently, is the most vital natural reserve which is crucial for life (Raji and Ibrahim, 2011). Safe, reliable, affordable, and easily accessible water supply is essential for good health (Howard and Bartram, 2003). Yet globally 1.1 billion people lack access to safe drinking water and 2.6 billion people lack access to adequate sanitation (WHO., 2004); Howard and Bartram, 2003). Access to safe drinking water can be accelerated by provision of affordable technologies for point of use (POU) water treatment and storage in combination with improved hygiene behavior. One of the most promising and accessible technologies emerging for POU drinking water treatment is Bio-Sand filtration (BSF) (Sobsey *et al.,* 2008).

Bio-Sand filters have been implemented in Kenya and over 20 countries worldwide. While the health benefits of using a Bio-Sand filter in terms of reduction of diarrheal disease have been fairly documented, little research has focused on the ability of this technology to remove other contaminants from drinking water that pose health concerns (Lantagne *et al.*, 2009). There are wide concerns in the developed and developing countries that drinking water resources are deteriorating in the long-term, both in quantity and quality (United States Environmental Protection Agency, 1995; Honisch *et al.*, 2002a).

Adverse agricultural activities are cited as the leading cause of surface and ground water pollution. Pollutants such as nitrates and pesticide are thought to pose serious risk to human

health (United States Environmental Protection Agency., 1995;Honisch, 2002) . Pesticides are developed through very strict regulation processes to work with logical certainty and minimal impact on human health and the environment.

But serious concerns have been raised about health risks resulting from occupational exposure and from residues in food and drinking water (Christos *et al.*, 2011). Many agrochemicals' compounds in use have a global and environmental contamination (Ahmed *et al.*, 2002). The potential for agricultural chemicals to cause endocrine disruption (ED) is an escalating worry, both in humans (Bretveld *et al.*, 2006) and in wildlife (Sparling *et al.*, 2001).

2,4-Dichlorophenoxyacetic acid (2,4-D) is the most commonly used pesticide in Ahero Irrigation Scheme (AIS) and it is a selective herbicide with highest toxicity to broadleaf plant (Garabrant and Philbert, 2002). It is used for post-emergence control of annual and perennial broad-leaved weeds in cereal, maize sorghum, rice and grassland as well as for control of aquatic weeds (Cox, 1999). In plants it functions by maintaining high levels of plant hormones auxine, resulting in overstimulation of plant growth and ultimately death. It also increases ethylene production and therefore acts as defoliating agent (Cox, 1999). Residues of 2,4-D can enter ponds and streams by direct application or accidental drift; by inflow of herbicide previously deposited in dry stream-bed, pond bottom or irrigation canals; run-off from soil or leaching through the soil column (Norris, 1981). Its production and degradation lead to creation of many compounds including chloro-phenols (Michałowicz, 2005) or dioxins (Bukowsk, 2004) that exert strong toxicity. 2,4-D has been

linked to several forms of cancer, primarily lymphoma and non-Hodgkins lymphoma as well as tumor of lungs, liver, kidney and brain in humans (Hoar *et al.*, 1986). Recently endocrine disruption has emerged as a threat to health of amphibian and human' population (Reeder *et al.*, 1998) and *Xenopus leavis* has been found to be ideal system to test teratogenic contaminants.

The present study is to compare survival, growth, developmental and teratogenic effects of 2,4-D on *Xenopus* embryo a claw-footed and tongue less frog native to Kenya.

Validation studies using compound with known mammalian or human developmental toxicity suggest that the predicted accuracy of FETAX approximate 85%, so it can be used to screen human developmental health toxicant (Courchesne and Bantle, 1985). Positive teratogens on the Xenopus leavis are also teratogen for humans.

Activated carbon owing to its high surface area and porosity is extremely efficient in removing varieties of pesticides from water (Francis and Lee, 1972). Granular activated carbon (GAC) is commonly used for removing organic constituent such as pesticides (Frank, 2000). AIS is considered in this study as a good example of a site where irrigated agriculture has negative impacts on the quality of domestic water supply. Hence BSF in series with GAC and Locally Activated Charcoal Bamboo (LACB) system was designed and tested in this study.

1.2 Problem Statement

The pressure to produce sufficient food for the fast-increasing population has resulted to increased use of agrochemicals compound such as pesticide (Osano *et al.*, 2000). However, after application; these pesticides enter the diverse environmental compartments: ground and surface water, soil, plants, and the atmosphere (Van den 1997). Only a small fraction of applied pesticides affects the target organisms and the rest often remains in the soil, contaminates surface water and groundwater resources, or mixes with the air (Topcu and Kirda, 2005). Irrigation practices play a major role in the accumulation of pesticide residues encountered in water resources.

Residues remaining in the soil are carried into water resources through the leaching of the soil profile and washed away through soil erosion (Topcu and Kirda, 2005). Extensive use of pesticides may pose serious concerns about health risks arising from the contact of farmers when mixing and applying pesticides or working in treated fields and from residues in food and in drinking water for the universal population have been on raise (Wilson , 2001;Pimentel, 2005).

In AIS extensive use of pesticide including 2,4-D may considerably pollute the surface water and cause ill health to the humans and ecosystem. The negative effects include some form of cancer, tumors of kidney, brain and liver (Hoar *et al.*, 1986) but little have been documented on its endocrine effects. The effects of the 2,4-D to aquatic species and riparian community is unknown and it is known have acute effects at the concentration of 70 ug/l or 0.007 mg/l in drinking water (USEPA., 1991). Therefore, treatment of water that is

contaminated with the pesticide is necessary but the available technology may be too expensive for the local people of Ahero. Possibility of an innovative and affordable technology that applies of principle of adsorption by activated carbon was studied. Activated carbon can effectively remove particles and organics from water (Clark, 1989). Therefore, in order to solve concerns over the presences of pesticide in treated water as is the case in AIS, granulated activated carbon and locally activated charcoal from bamboo was coupled with BSF.

Bio-Sand water filters are a technological adaptation of centuries old slow sand filtration process. While implementation of BSF exists in many sizes and varieties, most common design is intended for use in rural homes where naturally safe or treated water sources are not available (Palmateer *et al.*, 1999b). Several studies have been reported on the performance of the BSF in reducing bacteria, viruses and turbidity from feed water. Bacterial removals have been reported to vary from no apparent removal to 99% depending on operating conditions and filter ripening (Baumgartner, 2007;Duke, 2006) .

1.3 Justification of the study

Health effects of 2,4-D exposure is known to cause endocrine disruption effects and the most vulnerable members of the community to the exposure include children, pregnant women and breast-feeding mother. In the Millennium Development Goal 7c states that by 2015 half of the proportion of the population should have access to sustainable and safe drinking water.

Pesticide-induced chronic toxicity is also emerging as a serious public health concern. The toxicity manifestation that captures the greatest attention includes cancer, reproductive impairments and irreversible neurotoxicity (Albert *et al.*, 1992). 2,4-Dichlorophenoxyacetic acid (2,4-D) belongs to a group of chemicals known as phenoxy compounds, which are potentially toxic to humans (Boivin *et al.*, 2005). 2,4-D half-life ranges from 13 to over 300 days depending on the aquatic conditions (EPA., 1995) but it has been reported that it does not biodegrade efficiently a rapidly at concentration higher than 100 mg/l (Brillas *et al.*, 2000). It has been detected at level as high as 5 mg/l in the environment and directly applied as high as 50 mg/l in natural water for aquatic weed control (Lilienfeld, 1989;WHO., 1989) . The Kenyan Water Quality regulations 2006 for domestic use recommend nil mg/l of phenols (GOK., 2006). It has been reported that 2,4-D can be easily transported from field of application adjacent to wetland via surface run-off (WHO., 1984). In AIS the same pesticide has been found as high concentrations as 144 g/l was used per acre, hence it is assumed that high concentration of 2,4-D could be found in the study area.

Water pollution is any human-caused contamination of water that reduces its usefulness to humans and other organisms in nature (Carsel *et al.*, 2003). Contamination of water bodies with agricultural pesticides can pose significant threat to aquatic ecosystem and drinking water resources (Dabrowski *et al.*, 2002). However, the risk for aquatic community or for human health can often be substantially reduced by appropriate measures (Kreuger and Nilsson, 2001). These measures include cleaning water through a costly purification procedure (Carsel *et al.*, 2003).

2,4-D is a widely used pesticide found in surface and ground water all over the world. The fate for many pesticides under drinking water treatment conditions is a concern due to the potential adverse health effects as a result of consuming contaminated water, due to costly purification procedure of pesticide, Bio-Sand filters in series with activated carbon is a cheap technology that could remove both microbial and pesticides contaminant.

Xenopus laevis is a native species in Sub-Sahara Africa and have been used widely and successful to detect the teratogenic effects of environmental contaminants. In this work, with the support of the modified Frog Embryo Teratogenesis Assay-Xenopus (FETAX), a powerful and flexible bioassay for developmental toxicants (Dumont *et al.*, 1983; Bantle *et al.*, 1990; Vismara *et al.*, 1993; Bernardini *et al.*, 1994), the teratogenic potential of 2,4-D was studied.

Recently it was approved by Organization for Economic Co-operation and Development as the most reliable bioassay to investigate endocrine disruption by contaminants.

1.4 Objectives

The broad objective of this study was to determine whether Bio-Sand filter in series with activated carbon is effective in removal of 2,4-Dichlorophenoxyacetic acid in drinking water in Ahero irrigation scheme and evaluate the teratogenic effect of this pesticide by use of *Xenopus laevis*. The specific objectives were:

(i) To evaluate exposure regimes of 2,4-D by determining major domestic water sources for rice farmers in the scheme.

(ii) To investigate the teratogenic effects of 2,4-D by use of *Xenopus laevis* bioassays.

(iii) To assess effectiveness of Bio-Sand filter in series with activated carbon water treatment system.

1.5 Null Hypothesis

Ho: Rice farmers are not exposed to 2,4-D.

Ho: There are no teratogenic effects of 2,4-D on the *Xenopus laevis* embryos.

Ho: Bio-Sand filter in series with activated carbon water treatment system cannot produce portable drinking water.

1.6 Scope of the study

The Bio-Sand filter was assembled and bamboo-based charcoal was locally activated to enhance removal of pesticide. The variable to be studied included, finding out water sources for the people living around AIS and determining water quality of these sources. Also, the type and the amount of pesticide used by the farmers in Ahero Irrigation scheme during rice planting season were determined. The effectiveness of granular activated carbon and locally activated charcoal from bamboo charcoal was also assessed and finally the 96-h teratogenic effects of 2,4-D on *Xenopus laevis* embryos were investigated.

CHAPTER TWO: LITERATURE REVIEW

2.1 Health impact of pesticides

Portable water can be defined as water delivered to the consumer that can be safely used for drinking, cooking and washing (AWWA, 1990). Portable water must meet the physical, chemical, bacteriological, and radionuclide parameter (Montgomery, 1985). Several studies have shown that pesticides used in agricultural fields and volatilized into the atmosphere contaminate the domestic environment and may therefore be an important source of pesticide exposure to local populations (Loewenherz *et al.*, 1997; Ward *et al.*, 2006).

By their very nature most pesticides show a elevated degree of toxicity since they are intended to kill certain organisms and thus create some risk of harm. Therefore, pesticide use has raised serious concerns not only of potential effectson human health, but also about impacts on wildlife and sensitive ecosystems (Stoate *et al.*, 2001). An example of pesticide used in agricultural system is 2,4-Dichlorophenoxyacetic acid (2,4-D) (Fig 2.1).

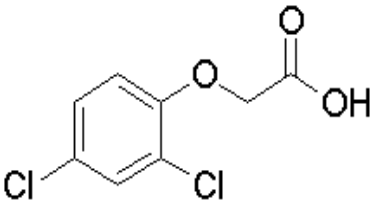


Figure 2.1: The chemical structure of 2,4-Dichlophenoxyacetic acid

Source: (Koesukwiwat *et al.*, 2008)

2,4 Dichlorophenoxyacetic acid is a strong acid and forms water-soluble salts with alkali metals and amines. Commercial 2,4-D products are marketed as the free acid, alkali and amine salts and ester formulations. 2,4-D itself is chemically stable, but its esters are rapidly hydrolysed to the free acid (CCME, 1995).

2,4-D is a post-emergent, selective control herbicide used to control broad leaf weeds in agriculture, forestry and in home lawns and gardens (Oliveira and Palermo, 1993). It is also used to control broadleaved aquatic weeds. Dioxin's impurities may be present in the technical product as a result of the manufacturing process (Health Canada, 1993). In plants 2,4-D functions by maintaining high levels of the plant hormone auxine, resulting in overstimulation of plant growth and ultimately death. It also increases ethylene production and therefore, acts as defoliating agent (Cox, 1999). 2,4-D inhibits root and shoot growth for both broad-leaved plants and grasses but in mammals and other species no mimic hormonal activity was observed (Osterloh *et al.*, 1983). It is identified that 2,4-D is in use up by the cells, passes quickly through the cell membrane, and is not metabolized (Bergesse and Balegno, 1995).

2.2 Environmental Fate and Health effects of 2,4-D acid

2.2.1 Environmental fate

Depending on the formulation, 2,4-D is moderately persistent pesticide with a half-life between 20-200 days (Bukowska, 2006). However, because 2,4-D has a very low soil-adsorption coefficient, it is likely to leach to aquatic environments, where its half-life

ranges from 13 to over 300 days depending on the aquatic conditions (EPA., 1995). Inappropriate aerial applications of 2,4-D can cause airborne drift of the compound to non-target environments and impact plants and animals (AAPCO., 1999).

2.2.2 Adverse health effect of 2,4-Dichlorophenoxyacetic acid

2,4-D have the potential to cause health effect when people are exposed to it at levels above the maximum concentration limit (MCL) of 7 parts per billion (ppb) or 70 ug/l for relatively short (acute) and long (chronic) periods of time.

2.2.3 Acute toxicity

Symptoms of acute oral exposure to 2,4-D includes vomiting, diarrhea, and headache, and confusion, aggressive or bizarre behavior. A peculiar odor is sometimes noted on the breath. Skeletal muscle injury and renal failure may also occur. Systemic toxicity is mainly linked with suicide attempts. Symptoms following dermal exposure may comprise irritation and inhalation exposure may lead to coughing and burning sensations in the upper respiratory tract and chest (Reigart and Roberts, 1999).

2.2.4 Chronic toxicity

Chronic exposure of 2,4-D has been linked to several forms of cancer, primarily lymphoma and non-Hodgkin's lymphoma, as well as tumors of the lung, liver, kidney and brain (Hoar *et al.*, 1986). Prolonged exposure to 2,4-D has been associated with reduced sperm counts and increased sperm abnormality in both humans and animals (Swan *et al.*, 2003).

It has also been strongly linked to extensive and profound developmental neurotoxicity and other fetal growth retardation in Laboratory animals and human cell cultures, suggesting similar effects to humans via exposure during pregnancy or early childhood (Rama *et al.*, 1995).

In Laboratory studies, 2,4-D exposure has led to thyroid hormone disruption, reduction in sex organ size, and disruption of metabolism generally. Human studies have also shown hormonal disruptions caused by 2,4-D. Also, several studies have concluded that exposure to 2,4-D caused chromosomal aberrations (breaks and/or rearrangement) in both animal and human subjects (Haasch, 2003). 2,4-D is considered a peroxisome proliferating compound, and has demonstrated the ability to disrupt gene expression and cell division, which could cause disruptions to organ system development as well as promote the replication of cancer cells primarily in the human liver (Haasch, 2003).

There is a concern that exposure to agricultural run-off could lead to the disruption of normal sexual development in amphibians and other wildlife through exposure to endocrine disrupting compounds. Disruption of normal sexual development can potentially place populations at risk by impairing their ability to reproduce.

2.3 Analytical method for 2,4-D

The analysis method used for the detection of 2,4-D is generally Gas chromatography (GC) (Santos *et al.*, 2000) and (Yu and Wells, 2007). Gas Chromatography/Mass spectrometer

(GC/MS) (Thorstensen *et al.*, 2000; Rodriguez *et al.*, 2005; Quintana *et al.*, 2007), or sometimes Capillary Electrophoresis (CE) (Miura *et al.*, 1999; Baggiani *et al.*, 2001; Farran and Ruiz, 2004). GC and some CE analyses have been shown to have adequate selectivity and sensitivity (Voos *et al.*, 1994). However, owing to the relatively low volatility of acidic herbicides resulting from hydrogen bonding of their carboxylic acids, these methods necessitate derivatization. Furthermore, owing to their polar nature; they are adsorbed onto the stationary phase, possibly resulting in peak asymmetry (Catalina *et al.*, 2000). Therefore, using a High-Performance Liquid Chromatography (HPLC) system, the necessity for derivatizations and perilous reagents can be obviated, while still generating steadfast experimental results. Moreover, HPLC is a more attractive methodology than GC because derivatization, when necessary, reduces method reproducibility by adding one extra sample-handling step before GC analysis (Tadeo *et al.*, 2000).

2.4 Bio-Sand Filter

The Bio-Sand Filter (BSF) is a household-operated slow sand water filter developed in the early 1990s; Dr Manz at the University of Calgary adapted the design of a traditional slow sand filter so that it could be operated intermittently and called it the Bio-Sand Filter (Buzuni, 1995;Palmateer, 1999).

2.4.1 Bio-Sand Filter Design

The design of the BSF can consist of concrete or plastic frames with locally available crushed rock as the filter media. The rock is crushed to two different sizes: a coarse layer and then a fine layer. The fine layer of crushed rock (sand) makes up the majority of the

filter, approximately 40 cm, and has an effective size of between 0.15 and 0.18 mm and a uniformity coefficient of 0.3 (Ahammed and Komal, 2011). The course layer of 5 cm has effective size particle between 1.18 and 4.75 mm and gravel layer of 5 cm with size particles of 4.75 and 12.0 mm (Figure 2.2).

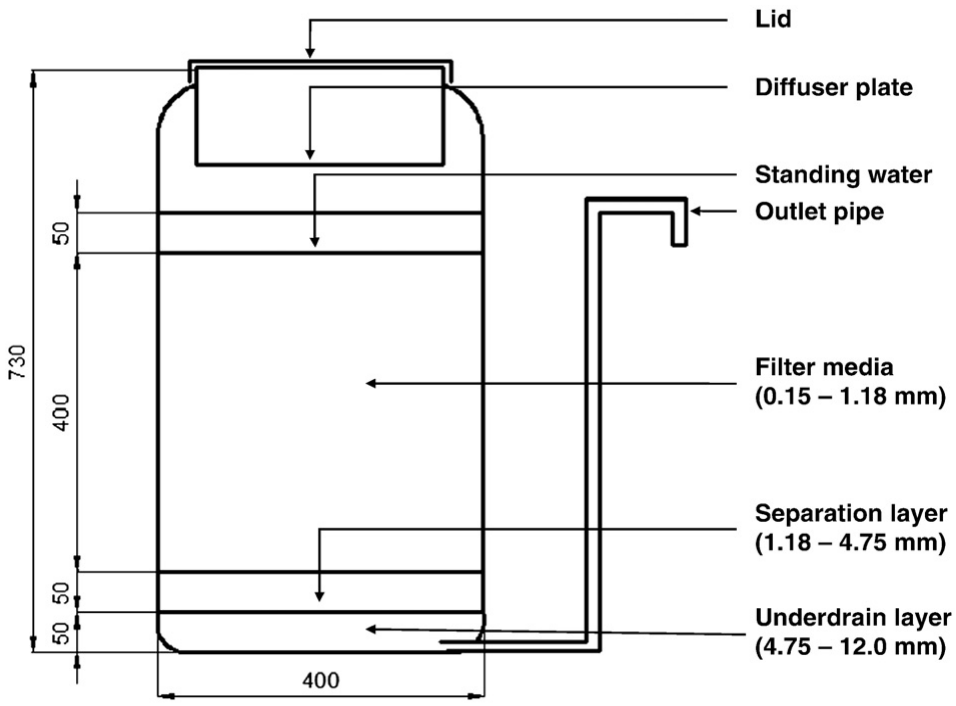


Figure 2.2: The Bio-Sand filter structure

Source: (Ahammed and Komal, 2011)

There are two principal mechanisms that govern the performance of slow sand filters; physical removal mechanisms and biological removal mechanisms. Physical removal occurs when particles there in the water are too big to pass through the filter bed. Biological removal occurs principally in the top layer of the filter in a biological film known as the

'schmutzdecke'. The schmutzdecke acts as both a fine filter to remove small colloidal particles as well as a biological zone that degrades soluble organics and destroys harmful pathogens (Weber-Shirk and Dick, 1997).

2.4.2 Bio-Sand Filters Operation

The BSF process works by passing raw water through a sand filter bed, where it is purified. The raw water at first enters the supernatant and then passes through the fine sand layer. On the surface of the fine sand layer, algae and other organic material from the raw water form a thin slimy zoogleal layer (Huisman and Wood, 1974b), known as the schmutzdecke from the German for "sludge blanket" (AWWA, 1990) will grow. The schmutzdecke is tremendously vigorous in feeding on dead algae and living bacteria from the raw water and converting them to inorganic salts. Concurrently, a major proportion of inert suspended particles are mechanically strained from the raw water (Huisman and Wood, 1974b).

As the water passes through the sand and deeper into the filter, beyond the schmutzdecke, a sticky zoogleal mass of microorganisms, bacteria, bacteriophages, rotifers and protozoa, known as the biofilm, forms and coats the sand particles. Organisms in the biofilm feed on adsorbed impurities and other organic material (including each other) carried by the raw water, which becomes attached to the sand through mass attraction or electrical forces of attraction. The organic matter is broken down into inorganic matter such as water, carbon dioxide, nitrates, phosphates and similar salts that are removed by the flowing water (Huisman and Wood, 1974b).

When the filter is not being filled, during the pause phase, oxygen is exhausted in the schmutzdecke and biofilm and the concentration of oxygen towards the bottom of the sand bed can become too low to support aerobic respiration. Live pathogens that reach this sand depth typically die as a result of the lack of oxygen (Ngai *et al.*, 2007) and leave the BSF with the effluent.

2.5 Activated carbon

Activated carbon is a solid, absorbent, black carbonaceous material. It is notable from elemental carbon by the absence of both impurities and an oxidized surface (Mattson and Mark, 1971). Due to extremely developed internal surface area and porosity, activated carbons have been used for thousands of years and have now become extremely versatile adsorbent. The major applications of activated carbon are the removal of species by adsorption from liquid or gas phase, to affect the purification or recovery of chemicals (Girgis *et al.*, 1994). It can be prepared from a large number of sources such as peat, coal, coconut, wood, tar, sawdust, and cellulose residues (Lambiotte, 1942).

Charcoal is produced by heating crushed carbonaceous substances to very elevated temperatures (600-900°C) followed by “activation” using steam or hot air to erode the internal surfaces of the product and thus increase its adsorptive surface area. The porous structure is largely developed during activation by means of an activation agent that reacts with the carbon. Such agents may include synthetic acids, bases, and other substances in a stream of activating gases such as steam (H_2O), nitrogen (N_2) or carbon dioxide (CO_2) (Smisek and Cerny, 1970). The spaces between the crystallites of activated carbon

constitute the micro porous structure with a large internal surface area of 250 m^2/g-2500 m^2/g. Carbonaceous adsorbents found greater use in the solution of environmental problems related to water purification (Smisek and Cerny, 1970; Hassler, 1974).

Granular activated carbon (GAC) and powdered activated carbon (PAC) are common sorbents. GAC adsorption is widely used technology for treating contaminated water, either working independently or coupled with biological degradations (El Diwani *et al.*, 2009 and Suffet and Wable, 1995). This is because of its ability to remove a wide range of organic and inorganic compounds that contribute to taste and odour in the finished water, adsorb regulated synthetic organic compounds and organic matter that result from the formation of disinfection by-products (DeWaters and DiGiano, 1990). It is made of multiple materials containing carbon; among them coal and coconut shells are the most common. The production of granular activated carbon is divided into two steps. The first step includes dehydration and carbonization. The second involves the activation of carbon through heating to the appropriate temperature (800 ℃ to 1000 °C) to burn away all degradable substances and create a richly porous structure with a large specific surface ratio, resulting in substantial adsorption capability (Hu, 2001 ;Yoshizawa, 2000).

2.5.1 Carbon Adsorption

Adsorption is defined as the enrichment of material or increase in the density of the fluid in the vicinity of an interface with a solid (the adsorbent) (Sing *et al.*, 1985). It may be classified as chemisorption or physisorption, depending on the nature of the interactive

forces. In chemisorption the transfer of electrons is significant and equivalent to the formation of a chemical bond between the sorbate and the solid surface. In physisorption the interactive forces are relatively weak (Sing *et al.*, 1985).

Naturally occurring organic matter (NOM) can adversely affect the adsorption capacity and adsorption kinetics of many micro pollutants such as organic compounds and pesticides. The NOM is almost always present in drinking water sources at mg/l levels (Quinlivan *et al.*, 2005). The two most important mechanisms for reduction in micro pollutant adsorptions are direct site competition and pores blockage. A study conducted by Matsui et al 2002 involved conducting adsorption experiments using two strongly adsorbing synthetic organic chemicals (SOC) (Simazine and Simetryn) and weakly adsorbing SOC (Asulam) in the presence and absence of NOM. A wood-based PAC and a coal PAC, both manufactured by Colgon Inc., were used in the experiment. This study found that the extent of adsorption for all three compounds shows adsorption decrease in presence of NOM. Therefore, NOM competed with the three SOC's for adsorption sites.

2.6 The *Xenopus laevis* use in the FETAX toxicity model

The genus *Xenopus* belong to the family Pipidae that is a group of tongue less, aquatic African clawed frogs. The frog is easy to raise in Laboratory. *Xenopus laevis* was found to be very useful for research related to embryogenesis due to several factors, including the ability to stimulate production of eggs any time of the year, external development of the embryos and large number of eggs (500-2700) produced by the female per spawning.

Furthermore, the embryo is all intact developing system, which undergoes evoluntary conserved events of cleavage, gastrulation, and organogenesis, comparable to those of other vertebrates, including mammals. Validation studies using compound with known mammalian developmental toxicity suggest that predictive accuracy of the *X. laevis* embryo test approximates 85%, so it can be used as an indicator of potential human health hazards (Bantle, 1989;ASTM., 1998).

Frog Embryo Teratogenesis Bioassay *Xenopus* (FETAX) is conducted at embryonic stage of development. This stage of development has demonstrated sensitivity and may provide information that is useful for estimating acute toxicity of a material. FETAX uses dual endpoints, mortality and malformation of embryos to asses developmental toxicity. Embryonic Teratogenic index (TI) is defined as 96-h LC_{50} (mortality) divided by 96-h EC_{50} (malformation). TI values higher than 1.5 signifies large separation of mortality and malformation concentration ranges and, therefore a large potential for all embryos to be malformed in the absence of significant embryo mortality (ASTM., 1998), while TI values less than 1.5 denote compound that are more embryo lethal, suggesting that they are co-effective teratogen (Johnson, 1981).

CHAPTER THREE: METHODOLOGY

3.1 Study area

Ahero Irrigation Scheme (AIS) which is considered as a study area is located 24 km southeast of Kisumu town in Nyanza province in western Kenya, 15 km south of the Equator at an altitude of 1,150 m above sea level (latitude 34.90^0 E and 34.97^0 E and longitude 0.11^0 S and 0.16^0 S) (Fig 3.1). It covers an area of square kilometers and has a population of about 7,891 people (HPCK., 1999).

The area has a relatively humidity of 65% due to its proximity to Lake Victoria. Local climate is characterized by three peaks of rains with an average annual rainfall of 1,000 – 1,800 mm and yearly mean temperatures vary between 17°C and 32°C. The first peak of rains occurs between March and July, with an average monthly rainfall of 150 – 260 mm. The other rainy season occurs in August. Short rains occur between September and October and have an average monthly rainfall of at least 125 mm. The dry period occurs between December and February (Githeko *et al.*, 1996).

The main economic activities include farming and subsistence farming of cultivated maize, sorghum, cassava, millet, and vegetables. Most people keep animals including cattle, goats, sheep, and poultry. Other activities include fishing due to its proximity to Lake Victoria and the Nyando River (Githeko *et al.*, 1996).

Nyando River is the major water source to the people living around the scheme. During rice planting season some residence obtain their water for domestic use from the irrigation canals from the scheme.

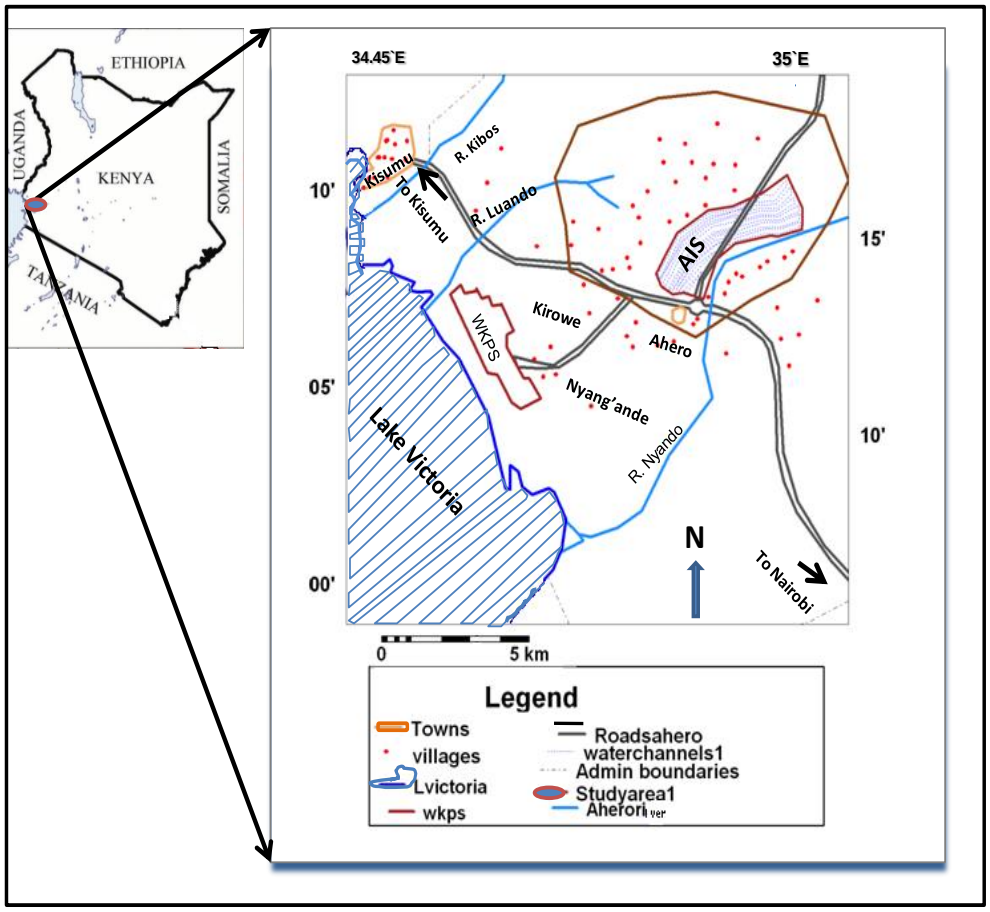


Figure 3.1: Location map for Ahero Irrigation Scheme
Source: Author, 17th April, 2013

Nyando District has outstanding agricultural activities. Rice farming is the main cash crop in the region and it covers approximately 2404 hectares. The residents' main source of water for domestic water are both surface water and groundwater through Nyando River, boreholes, few ponds and irrigation canals during rice planting season. In AIS, rice planting

is done throughout the year but in rotation (spatial) due to scarcity of water from Nyando River.

3.2 Materials

3.2.1 Laboratory analysis

1. The following are chemical reagents that were used for Laboratory analysis of 2,4-Dichlorophenoxyacetic acid.

Chemicals: 2,4-Dichlorophenoxyacetic acid (Bellefonte, 99.8% purity, USA), was used as the standard and for spiking the sample, methanol (Aldrich, 99.8% purity, England), that was used as a solvent, Acetonitrile (Fluka, 99.5% purity, Switzerland), that was used as mobile phase during analysis with HPLC.

2. The following are chemical reagent and salts that were used for Laboratory analysis to investigate the teratogenicity of 2,4-D by use of *Xenopus laevis* bioassays.
For induction of spawning, Human Chorionic Hormone (Organon, Oss, Netherlands), was used. Analytical grade solvent dimethylsulfoxide (DMSO) ($CH_3.SO.CH_3$), (Merck, 99.9% purity, Germany), was used as solvent in dissolving 2,4-D.

Salts: FETAX solution was prepared as 625 mg Sodium chloride (Merck, 99% purity, Germany) 330 mg potassium chloride (Ranbaxy, 99.8% purity, New Delhi), 96 mg sodium hydrogen carbonate (BDH, 99.7% purity, England) 5 mg Magnesium sulphate (Fluka, 99.5 % purity, Switzerland), 15 mg Calcium chloride (Merck, 99.9% purity, Germany), 60 mg calcium sulphate (Fluka, 99% purity ,Switzerland), L-Cysteine (Fluka, 99.5 % purity, Switzerland), all analytical grade per liter of distilled water.
Test organism: *Xenopus laevis* was obtained from Ahero ponds.

3.2.2 Bio-Sand Filter construction

The following material were used during Bio-Sand filter construction

One plastic container with lid was the filter container about 60cm tall, piece of (2 cm) PVC pipe, three elbows 3/4-inch (2 cm) PVC fittings, two elbows 3/4 inch (2 cm) threaded, two 3/4 inch (2cm) threaded, PVC connector. Small stones (marble size), enough to fill half of the supply container, Coarse sand or small pebbles, enough to fill 1/4 of the filter container and fine sand enough to fill 2/3 of the filter container.

3.2.3 Materials used for preparation of locally activated charcoal from bamboo (LACB)

Calcium chloride (Merck, 99.9% purity, Germany), Kiln and crusher.

3.3 Apparatus

Combo by Hanna pH, temperature and EC (CE HI 98129, Japan) apparatus was used to measure electrical conductivity, pH and temperature during water sample collection. Turbidity meter was used for measuring turbidity of the water samples.

3.4 Experimental Methods

3.4.1 Primary data collection

Interviews were conducted and structured questionnaires were administered to stakeholders (Agro-chemist stockiest, National Irrigation Board and local people). All the entire agrochemical stockiest were interviewed while participants from the local community were selected using systematic sampling (Kothari, 2009). The research assistants were trained on how to administer the questionnaires correctly. It consists of selecting the first house along the road and/or path from the irrigation scheme as the first participant and after that every 10^{th} house was interviewed. Data collection was obtained from 72 households living within Ahero irrigation scheme.

(i) Sample size

Sample size was determined from the formula proposed by Yamane cited by Isreal (2009), which state that:

$$n = \frac{N}{1 + N(e)^2}$$

Where: n= sample size

N= target population size

e= level of precision (sample error)

Therefore, N=89 stakeholders and e=5%

$$n = \frac{89}{1 + 89(0.05)^2}$$

$$n = \frac{89}{1.2225}$$

N=72

The returned questionnaires were validated through focused group discussion in Ahero Irrigation Scheme. The obtained information was used to give the data on the types, amount of the pesticide used around the scheme and also to identify the water sources. The farmers' questionnaires were translated into local dialect for better understanding (Appendix 1). The agrochemical stockiest and NIB questionnaires consisted of questions on the type of pesticide and the amount applied per acre (Appendix 2).

3.4.2 Secondary data collection

Secondary information was obtained from National Irrigation Board, ministry of water and agriculture. All these institutions availed information from their reports on the soil type or texture, topography of the area, population, vegetation cover, and crops planted.

3.4.3 Water Collection

Representative water sample was collected from an irrigation canal that is currently used for domestic purpose. Sixty liters of water were drawn from the site and taken to University of Eldoret where analysis was carried out. Field survey using structured questionnaires and informal interviews was used to collect information on their major water sources. The

sample water was spiked with 2,4-D at several concentrations to assist in evaluating the efficiency of both granular activated carbon and locally activated charcoal from bamboo.

3.5 Adsorbent materials and reagents

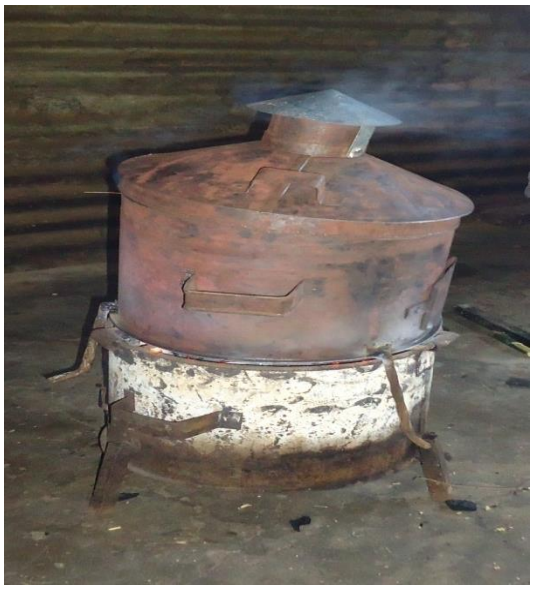
3.5.1Plant material

Bamboo plant *(Arundinaria alpina)* was obtained from Tree Nursery at Moi University Main Campus and the plant was identified using dichotomus key.

3.5.2 Method for activated charcoal preparation from Bamboo

Bamboo was cut into small pieces of 20cm and 3-5cm wide to allow uniform burning in the furnace and then dried under the sun shine for seven days (Kearns., 2008), the dried material were then placed into an oil drum and that was then lit from the bottom. Once the materials inside the drum were fully ignited and the water in the carbon source had evaporated, the drum was sealed to commence the anoxic combustion procedure. Over the next two to three hours, the material was successfully carbonized and charcoal was formed (Cobb *et al.*, 2012).

After charcoal was produced as described above, the prepared charcoal was then washed with distilled water to remove remaining organic material content and finally dried in an oven at 110^0C (Mishra and Patel, 2007). The materials were broken into smaller pieces without crushing them down to fine powder. The size of activated charcoal was between 0.5 mm and 1.0 mm; this was obtained by the use of sieves. Once this step is completed, the pieces were soaked in a 25% solution of $CaCl_2$ for 24 hours. They were then rinsed thoroughly and placed in an oven at approximately 100°C (Cobb *et al.*, 2012). The plate 1 below shows a fabricated kiln for activating bamboo strand which was constructed by using 2mm thick of steel metal.



Plates 3.1: Fabricated kiln

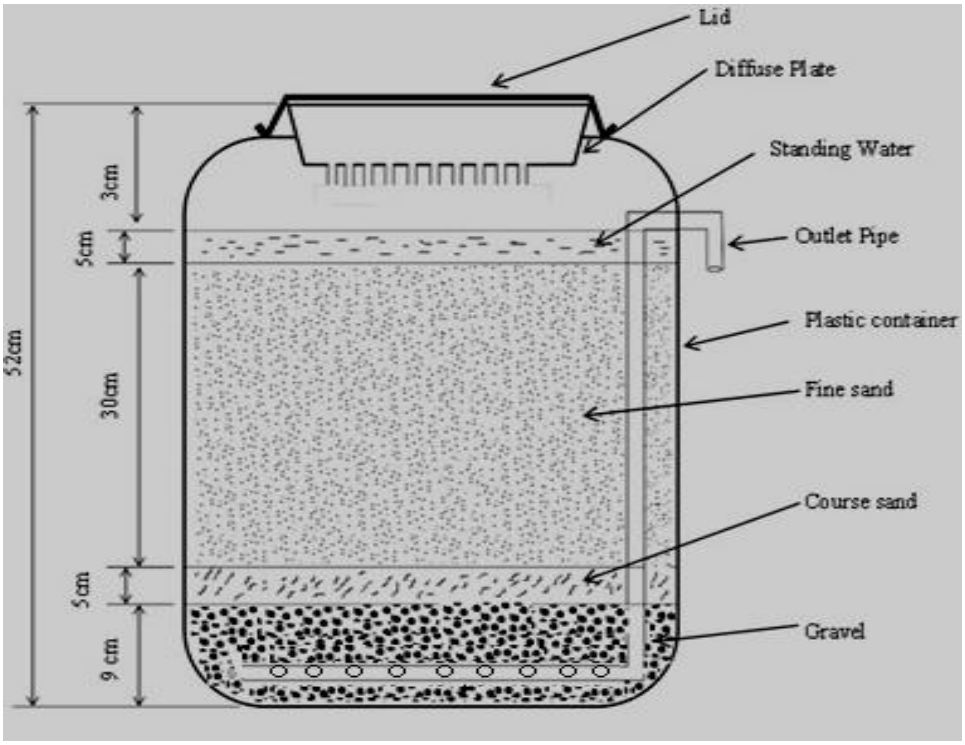
Source: Author, 20th August 2012

Plates 3.1: Activated charcoal from bamboo

Source: Author, 20th August 2012

Method used in Biosand instalation

The sand was washed several times using tap water until the wash water became clear according to the standard procedures for BSF developed by Manz (2007). Plastic container (75 liters) obtained from the local market was used for constructing the BSF. The container was first cleaned with tap water and was filled with 9 cm deep under drain gravel (12.0 mm size), 5 cm of coarse sand (4 mm size) separation layer and 30 cm of sand (between 0.6-1 mm size) layer in succession (Huisman, 1974 ;Visscher, 1987) as shown in the Figure 3.2. Water was present in the container before loading the filter media to avoid any occurrence of air spaces. The outlet pipe was set in such a manner that the water depth of 5 cm was maintained over the filter media. A plastic diffuser plate was placed on the lip of the filter to avoid disturbance to the top layer of sand during daily charging of the filter with raw water. The filter was fed with 40 liters of water daily; 20 liters in the morning and 20 liters at night each day for 21 days before using it to allow the maturation of the filter (formation of biological film).



Plates 3.2: Schematic diagram of Bio-Sand filter

Source: Author, 15th September 2012

3.7 Fixed bed column for 2,4-D removal

Fixed bed column was used for 2,4-D adsorption. The bench scale plant consisted of six columns of 500 mm height each and 20 mm inner diameter. The six columns were filled with activated carbon. The first three were filled with granular activated carbon and the last three to the activated charcoal from bamboo. All columns were filled with 100 grams of activated carbon and the particle size for all were between 0.5 mm to 1 mm. Prior to the experiment, distilled water was passed through the column to rid the column of impurities and air bubbles. Water from Ahero irrigation scheme was purposely used to determine the adsorption efficiency capacity of adsorbent as the surface water may contain multiple organic impurities probably present in water from irrigation canal that may interfere with adsorption (Anthony, 2005) and (Matsui *et al.*, 2002) by reducing available pesticide adsorption sites available. Water was spiked at 5 mg/l, 50 mg/l and 200 mg/l nominal concentration in order to determine the efficacy of both GAC and LACB on the removal

of pesticides and also due to low sensitivity of the HPLC to low concentrations, explaining why environmental relevant doses that people are exposed to were not used.

Concentration of 5 mg/l, 50 mg/l and 200 mg/l that was used represented different toxicity levels of 2,4-D in the *X. laevis*. For each of the three concentrations, the contact time was varied from 30 minutes, 60 minutes and 90 minutes. This was to determine the maximum adsorbing capacity of adsorbent of pesticide from contaminated water. Contaminated water was also passed overnight through Bio-Sand filter for every concentration. Three samples were collected for every concentration. The fixed bed column bench scale setup (Plate 3.3) was done because of its large internal surface area and pore volume (Prakash *et al.*, 1994) and for Bio-Sand filter use; fixed bed column was applicable. Water was left in Bio-Sand filter overnight because the filter usually has 60 liters of standing water and the water that is collected for usage every day is the displaced water from the container.

Plates 3.3: Laboratory bench scale experiment

Source: Author, 10th March 2013

3.7.1 Experimental layout indicating the pesticide removal potential of BSF filter

The raw water that was obtained from AIS was spiked with pesticide to simulate field contamination situation. The spiking was done at different concentrations at 5 mg/l, 50 mg/l and 200 mg/l this was done to assess the removal potential of 2,4-D by the Bio-Sand filter. The spiked water was then passed through Bio-Sand filter and left for 24-hrs then collected the following day. The sampled water was filtered and stored in vials for analysis (Figure 3.3) and the remaining was passed through activated carbon as shown in flowchart (Figure 3.4).

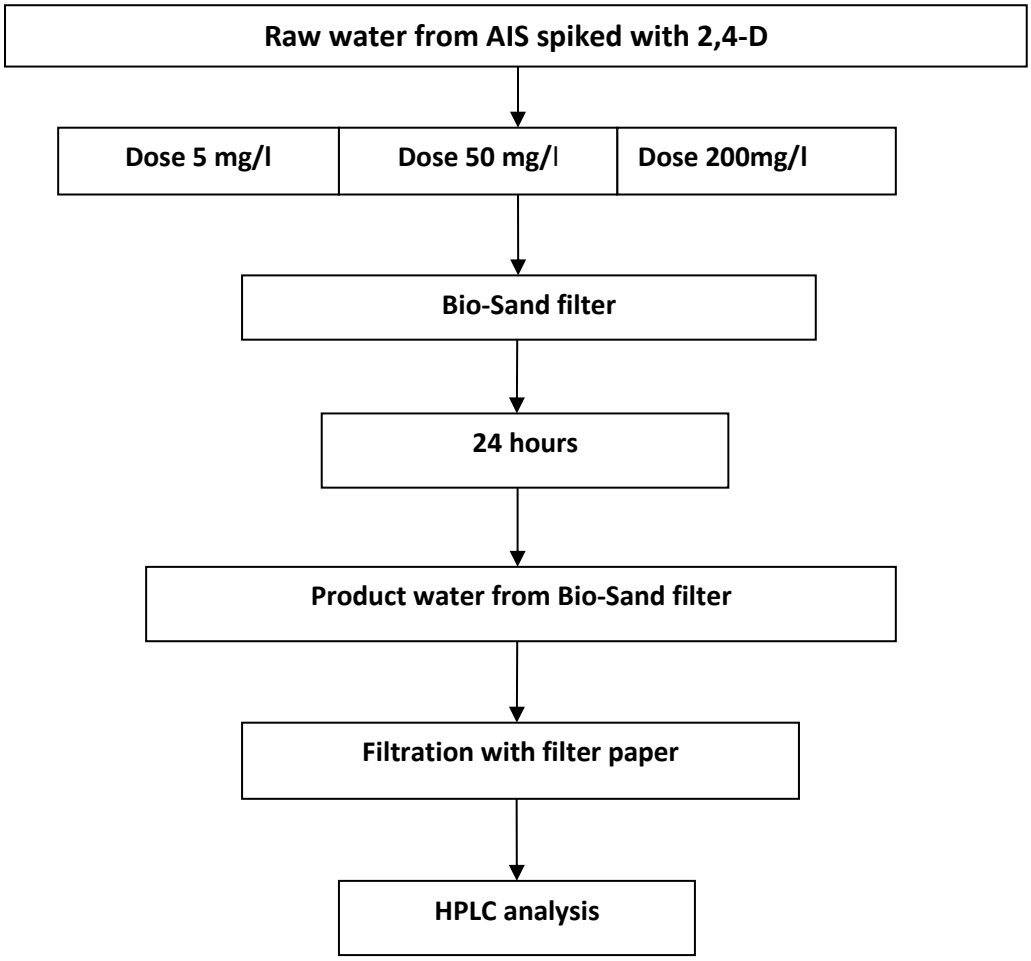


Figure 3.1: Experimental set up for Bio-Sand Filter removal assessment

3.7.1 Effect of contact time and the dose on removal of 2,4-D using LACB and GAC

An aliquot of emergent from BSF was passed through granular activated carbon and locally activated charcoal from bamboo charcoal each. The contact time was 30, 60 and 90 minutes for every concentration. Also the sampled water after each contact time was passed through filter paper and stored in vials for further analysis as shown in the flow chart below.

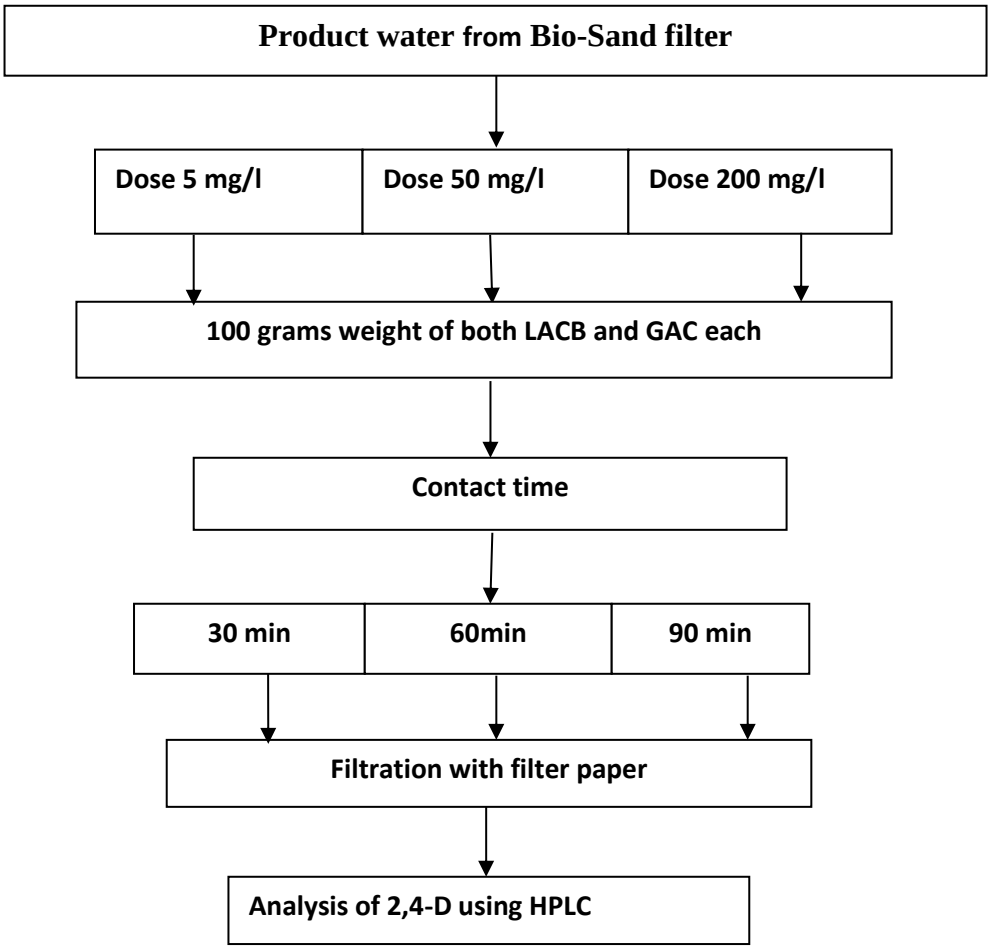


Figure 3.2: Experimental set up for GAC and LACB assessment

3.8 High-performance liquid chromatography 2,4-D analysis

Analysis was carried out at the Kenya Tea Research Foundation (TRF) in Kericho using the HPLC system of a Shimadzu (Kyoto, Japan) model HPL-20A liquid chromatography

with a UV detector at a wavelength of 235 nm. Twenty microlitres were injected for all standards and final water samples. The mobile phase was a mixture of 4% acetic acid/acetonitrile (60:40). Analytical separation was achieved on an Aqua C18 A Hypersil ODS (125 mm × 4 mm, 5 mm) column was used for separation. The flow rate was 1 mL/min (pressure about 2100 psi) and the temperature was ambient.

Plates 3.4: HPLC used for sample analysis

Source: Author 22nd July, 2013

Plates 3.5: Pipetting the sample to the vials for HPLC analysis

Source: Author 22nd July, 2013

3.9 Test animal; housing, breeding and harvesting

Embryos of *Xenopus laevis* (East African clawed frogs) were the standard test organism for the bioassay. Adult frogs were obtained from Ahero Pond and transported to be raised in University of Eldoret's Biotechnology Laboratory. The frogs were acclimated for six weeks before experimentation. They were housed and maintain in 20-gal aquaria. Frog cages were filled with dechlorinated tap water (aerated 1 week to a depth of 15 cm). Frogs were fed a diet of Xenobites ®. The frogs were fed twice a week and aquaria water was replaced at the time of feeding. The aquaria were maintained at a 12:12 h light: dark regime at room temperature of 24^0C (ASTM, 1998).

3.9.1 In vitro fertilization

Adults' frogs were selected randomly for each FETAX run. At least three different male/female pairs (one per cage) of mating *X. laevis* were placed in 0.3 × 0.3 × 0.3 m cages half-filled with FETAX solution. The bottom of the cages was separated about 3cm from the frogs by use of Perspex floor with 1-cm-diameter holes. The holes allowed the eggs to sink below hence prevented the adult frogs from eating them. The medium was aerated and maintained at 23^0C. Human Chorionic Gonadotropin (HCG) was used to induce mating in adult frogs and was administered by subcutaneous injection (1 ml tuberculin syringe into dorsal lymph sacs between the lateral line and spine of the frogs). Males and females were injected with 500 IU and 1000 IU of HCG, respectively (ASTM., 1998). The frogs were placed into 3 glass aquaria filled with 10 liters of FETAX solution. The frogs were checked approximately 14 hrs later for evidence of successful mating.

Fertilized eggs were harvested with sterile plastic pipettes and placed into a beaker of FETAX solution. The jelly coat surrounding the egg was removed by gentle rotation (2-3 minutes) in a 2 % solution of L-cysteine (w/v) adjusted to a pH of 8.00-8.10 with 1N NaOH. After membrane removal, the L-cysteine was quickly drained, and the eggs were rinsed four times with FETAX solution. Embryos of stage 8 (midblastula) to stage 11 (early gastrula) were selected from assay using Normal tables (Nieuwkoop and Faber, 1975).

3.9.2 Experimental Preparation

Before each FETAX run, all glass wares and plastic wares were soaked in detergent for 10 minutes, washed 10 times with tap water, solvent washed (20% HCL) for 30 minutes and finally rinsed 10 times with tap water and distilled water and oven-dried at 200^0C. The 2,4-D was weighed on an analytical balance and dissolved in 1ml DMSO then diluted into FETAX solution to make a sub-stock solution. All bottles were shaken before each static renewal to ensure adequate mixing. Test solution was prepared fresh at the beginning of every 24-h. Because 2,4-D is quickly biodegraded in aqueous environment, fresh 2,4-D stock solution were prepared every 48 hrs. Aluminum foil was placed over all bottle for

each 2,4-D experiments to prevent photo degradations. Three replicates were used for each test solution. The incubation temperature was maintained at 24± 2 ^{o}C for FETAX bioassay.

Normally developing embryos between stage 8 (blastulae) and stage 11 (gastrulae) were randomly selected after harvesting of the newly fertilized eggs. The embryos in 60 mm by 10 mm glass petri dishes containing FETAX solution were placed into identical glass petri dishes containing test solutions. The bioassay required static renewal of all test and control solutions at 24, 28 and 72 h. Dead embryos were removed from petri dishes at each static renewal, and the number of dead embryos was recorded. The experiment was terminated at 96-h.

Plates 3.6: Petri dishes in incubator
Source: Author 22nd July, 2013

Plates 3.7: Eggs in the petri dish
Source: Author 22nd July, 2013

3.9.3 Testing procedures for effects of 2,4-D

Studies were carried out for 96 h at 24±2^{o}C and renewed daily with respective test solution according to the standard FETAX protocol (ASTM., 1998). Three definitive tests were conducted on the test material in random block design with embryos from each of the three pairs forming a block. Each block comprised duplicate tests and in each test 20 embryos were exposed to 5 mg/l, 25 mg/l, 50 mg/l, 100 mg/l, 120 mg/l, 150 mg/l, 170 mg/l, 200 mg/l, 220 mg/l and 240 mg/l nominal concentration of 2,4-D. The actual concentrations

were concentration of 2,4-Dichlophenoxyacetic acid in Petri dishes (Plate 3.7). 2,4-D was dissolved using DMSO and FETAX was used to serially diluted it to make stock solution. Simultaneously, three controls dishes of each FETAX for each three couple's couple A, couple B and couple C. All the Petri dishes were randomly placed in an incubator set at 24±2^{o}C (Plate 3.6). Daily renewal was achieved by removing the test solutions with a Pasteur's pipette whose mouth was smoothen by heating over a flame to prevent it from injuring the embryos. Mortality was assessed daily and dead organisms were removed as required while recording the number of the live ones. At the end of 96-h incubation period the surving embryos were fixed in 5 % formalin (ASTM., 1998). The 96-h LC_{50} and the 96-h EC_{50} were determine by probit analysis after Abbott's (1925) adjustment for mortalities and malformations in the control Y=100*(C-T)/C, where Y= percent response C= percent not responding in FETAX control; T= percent not responding in the test solution and the response was considered as growth retardation, mortality and malformation. Malformations were then examined under a binocular-dissecting microscope at a magnification of 10X. An abnormality was scored regardless of the severity of the abnormality, for example, a slight tail flexure, large flexure or extensive tail flexures were all scored as tail flexure. The frequency of each type of malformation at each concentration was recorded. The reported incidence of each type of malformation was an Abbott's (1925) adjusted incidence for the similar type of malformation occurring in the FETAX control. Head to tail lengths of the embryos were measured with help of an ocular micrometer. However, for the flexed larvae, length measurements were taken along the curvature of the notochord. The Atlas of Abnormalities was used as a guide to distinguish between normal and abnormal embryos and determine the different kinds of Abnormalities (Bantle *et al.*, 1990).

3.10 Data analysis

3.10.1 Questionnaires

Each set of questionnaires, the responses from the farmers, stockiest and NIB were coded and keyed in the statistical package of social scientist (SPSS) to analyze data. Spearman's

rank order correlation (r_s) was used to show whether and how strongly pairs of variables were related where r_s value varies between -1 and 1($-1 \leq r_s \geq 1$).

3.10.2 Teratogenicity of 2,4-D

Collected data were statistically analyzed using descriptive statistics in the statistical package for social scientist (SPSS) version 16 and Microsoft office Excel 2007®. The Teratogenic Index (TI) was obtained by dividing the LC_{50} by EC_{50} and used for quantifying the degree of Teratogenicity.

3.10.3 Physico-chemical and Laboratory analysis data

The results consisting of in-situ and ex-situ analyses of electrical conductivity, temperature, turbidity, pH was analysed using (SPSS 16 for windows student version) and Microsoft Office Excel 2007 ® was applied to analyze physico-chemical and laboratory data.

3.10.4 Removal of 2,4-D using BSF, GAC and LACB

Collected data from removal of 2,4-D using Granular Activated Charcoal and Locally activated charcoal from bamboo were statistically analyzed using t- test in the statistical package for social scientist (SPSS) version 16 and Microsoft Office Excel 2007® for comparing the efficiencies of both GAC and LACB. Also, the data collected from removal of 2,4-D using Bio-Sand filter was analysed using descriptive analysis using Microsoft Office Excel®.

CHAPTER 4: RESULTS

4.0 Introduction

This chapter focused on the results of the study. It is presented in four sections; the water source to the household living around Ahero Irrigation Scheme (AIS), the type of weed control used in rice farming in AIS, the teratogenic effects of 2,4-D to wild exposed *Xenopus laevis* and the removal of 2,4-D using Bio-Sand filter (BSF), Granular Activated Charcoal (GAC) and locally activated charcoal from bamboo (LACB).

4.1 Socio-economic characteristics of households

The general information about socio-economic characteristics of household heads determined was as follows: gender, education level and the length lived in the scheme. The gender distribution of the household is given in Table 4.1. The level of education is presented in Table 4.2 and the length of period lived is presented in Table 4.3.

Slightly more than two thirds (68.1%) of the interviewed household were female compared to close to a third (31.9%) who were male.

Table 4.1: Sex of household head

Gender	Frequency	Percentage
Female	49	68.1
Male	23	31.9
Total	72	100

Among household heads interviewed, 75.0% attained primary school level, 16.7% secondary school level, 4.2% tertiary college 2.8% university and only 1.4% vocational/village polytechnic. Three quarter (75%) attained primary level maybe due to poverty and early marriages Table 4.2.

Table 4.2: Level of education of household heads

Level of education	Frequency	Percent
Primary	54	75.0
Secondary	12	16.7
Vocational/Village polytechnic	1	1.4
Tertiary College	3	4.2
University	2	2.8
Total	72	100.0

Majority of the household 87.5% have lived around the scheme at the time of the project more than two years, 8.3% less than six months, 2.8% between one to two years, while 1.4% between six months to one year. The results indicate that majority of the household are permanent residents of Ahero Table 4.3.

Table 4.3: Length of time the household head have lived around the Ahero irrigation scheme

Time	Frequency	Percent
Less than 6 months	6	8.3
6 months to 1 year	1	1.4
1 to 2 years	2	2.8
More than 2 years	63	87.5
Total	72	100

4.2 Domestic water sources

The respondents were asked the source of water for their household and the results are given in Fig 4.1. The results shows that 47.2% of the people living around Ahero irrigation scheme draws their water for drinking and domestic needs from lined improved well, 22.2% from irrigation canal, 16.7% from Nyando river, 4.2% for open well dug in river

bed/wetland and tap water respectively, 2.2% from pump bore and rain water reservoir respectively.

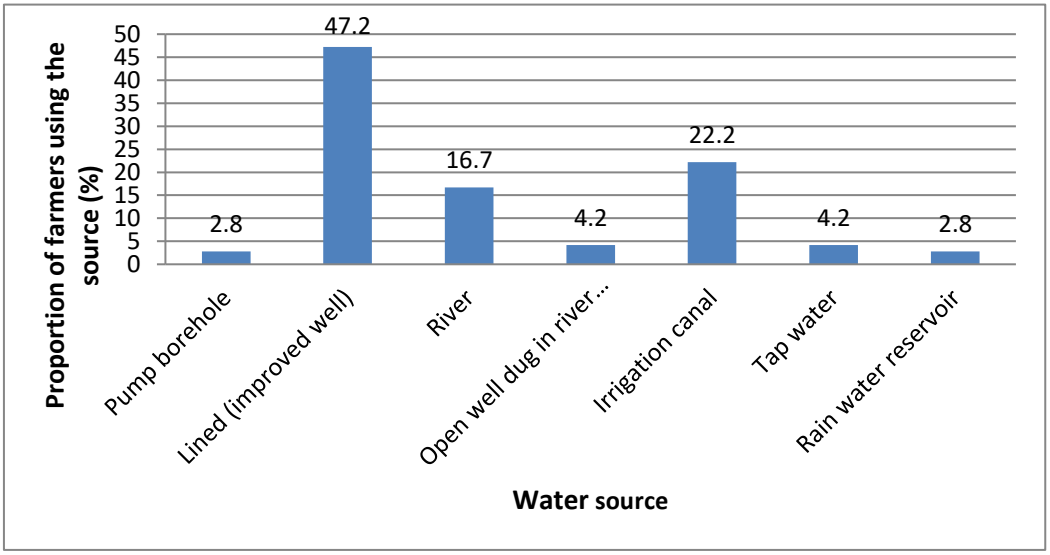


Figure 4.1: Source of water at the community level

The results indicated that a significant number of households draw their water supply from irrigation canal (22.2%) (Plate4.1) and sixteen percent from River Nyando which has a high potential of contamination from agricultural runoff, with pesticides residues.

Plates 4.1: Woman fetching water from irrigation canal for domestic use

Source: Author, 5th June 2013

Further when respondent was asked on the preference of the water source their results in (Fig 4.2) shows that 37.5 % of the people living around the scheme prefer water from lined improved well, slightly more than a third (36.1 %) from irrigation canal 18.1% from the river, 4.2 % from tap water, 2.8% pump borehole and 1.4% open well dug in riverbed/wetland.

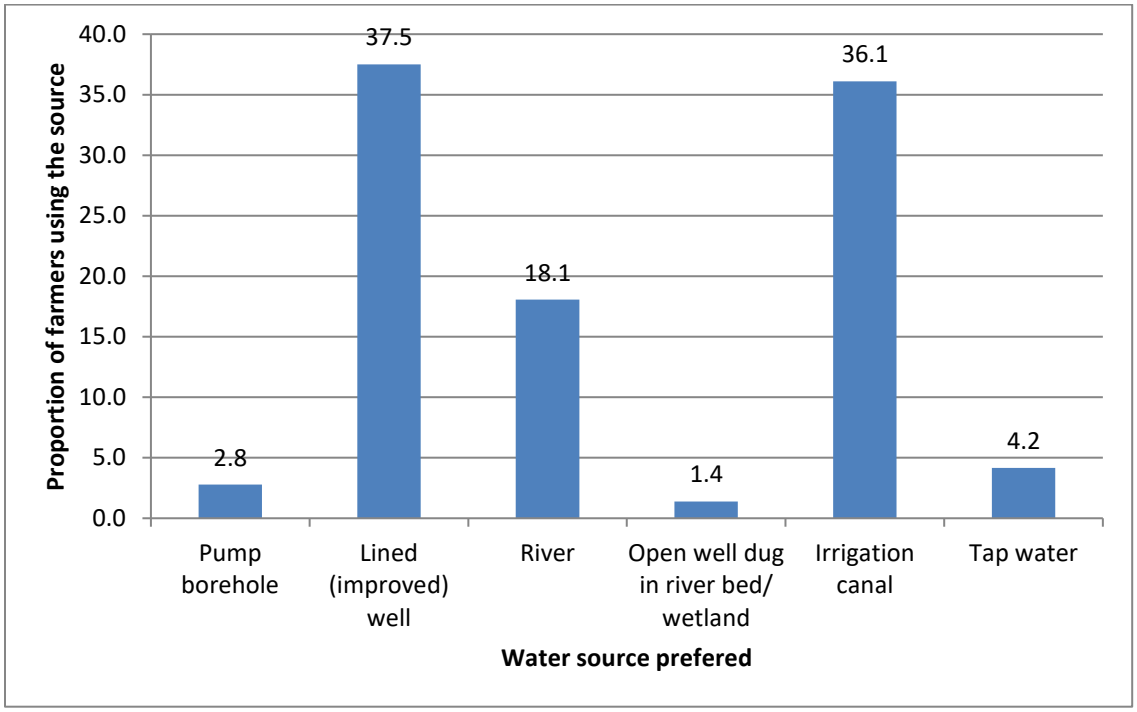


Figure 4.2: Preference of different water source at the community level

Slightly above a third of the household that preferred to draw their water from irrigation canal said it was less salty and closer to them. The problem is that the turbidity is much higher than the improved well. Its only problem was a higher turbidity. High turbidity issue was solved by use of a coagulant (Alum) as stratified by (16.7%) of interviewers. Common alum is hydrated potassium aluminum sulphate $KAl(SO_4)_2.12H_2O$ (Austin, 1984), which allows the suspended particles to settle down leaving the water clean and clear.

4.2.1 Type of water treatment used by the community

The study also focused on type of treatments the local community uses for their drinking water and presented in Fig 4.3. The study established that almost half (45.8 %) of the people are chlorinating their water, 22.2% do nothing to their water for drinking, 16.7% uses coagulant, 12.5% filters with a cloth and only 2.8 % boil their water before drinking.

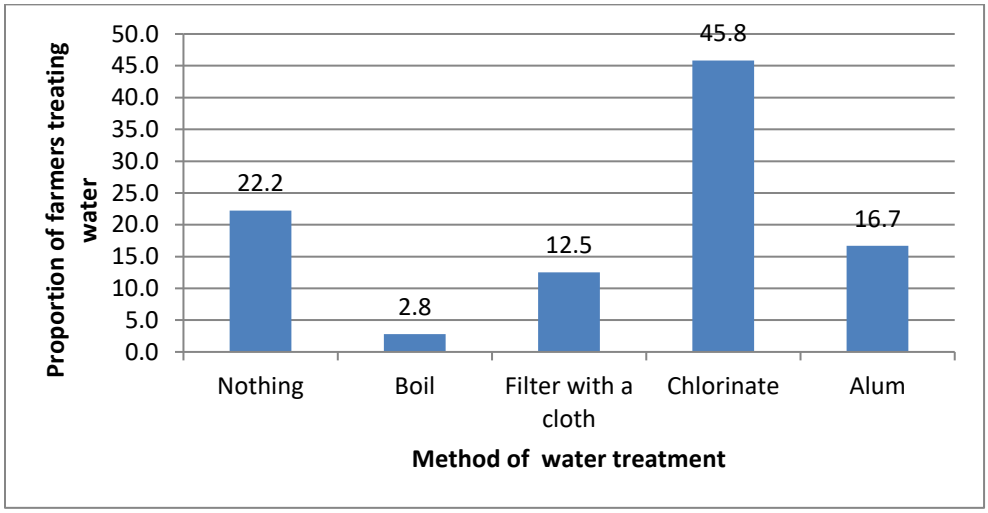


Figure 4.3: Proportion of different method of water treatment used by the farmers used in AIS

The household that uses alum (16.7%) for their drinking water treatment was perceived to obtain their water from either irrigation canal or river where the water turbidity is high. While those who do not use any water treatment was perceived to obtain their water from lined improved well, pump borehole or tap water due to low turbidity of the water.

4.2.2 Relationship between water source and the type of water treatment

The source of water was perceived to influence the type of water treatment and Spearman's coefficient of correlation was used to establish any relationships and the results are given in Table 4.4. It shows that 13.9 % of the people who obtain water from lined improved well do not treat their water, 4.2% filter their water with cloth and 25% chlorinate their water. Spearman's coefficient of correlation (r_s) revealed that there was positive correlation

between the two variables (r_s=*0.145*, n=72, p=0.224>0.05). As r_s is positive, it implies that the type of treatment given to water depend from it source of the water

Table 4.4: Cross-tabulation of water source and method of drinking water treatment

Water source		**Method of treatment of drinking water used**					
		Nothing	**Boil**	**Filter with cloth**	**Chlorinate**	**Alum**	**Total**
Pump borehole	%	-	1.4	-	1.4	-	2.8
Lined (improved) well	%	13.9	1.4	4.2	25	2.8	47.2
River	%	-	-	5.6	5.6	5.6	16.7
Open well dug in river bed/ wetland	%	-	-	-	4.2	-	4.2
Irrigation canal	%	4.2	-	2.8	6.9	8.3	22.2
Tap water	%	4.2	-	-	-	-	4.2
Rain water reservoir	%	-	-	-	2.8	-	2.8
Total	%	22.2	2.8	12.5	45.8	16.7	100

Symmetric Measures

a-not assuming the null hypothesis

a-using the asymptotic standard error assuming the null hypothesis

c- based on the normal approximation

	p-Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig
Spearman Correlation	.145	.123	1.227	.224[c]
N of Valid Cases	72			

4.3 Pesticides application in Ahero Irrigation scheme

This study established that (52.8%) of the farmers used hand-weeding and 20.8% of them use 2,4-D for the weeds control (Figure 4.4). Slightly more than half (52.8 %) of the farmers use mechanical method of weeding and the remaining 47.2 % use various chemicals. It was established that 20.8% of the farmers applied 2, 4 D herbicide to their rice field at the rate 144 g/l 2,4-D is used per acre. 12.5% of the farmers used Sevian while 8.3% of the farmers used Di-amine. 5.6 % of the farmers used Murhamine and Dicopur. All their herbicides,

were applied with the sticker, wetter or sprayer with 0.5 liters of water to enhance efficiency of herbicides to the rice.

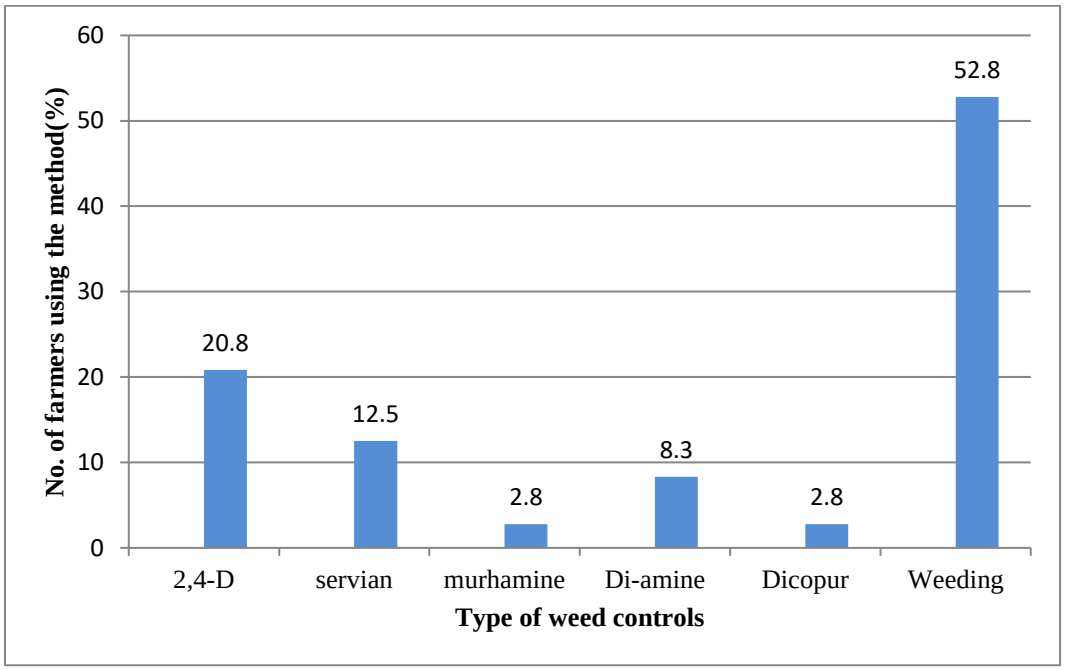


Figure 4.4: Weed control method used by rice farmers in Ahero irrigation scheme

4.5 Embryolethal effects, Teratogenicity and Growth retardation

Embryolethal effects of 2,4-D was assessed using *Xenopus laevis* embryos and the standard FETAX media was used as a control for comparing survival and mortality with test media. The survival value was 82.50±5.58 % (SE) and mortality rate of 17.20±5.56 % (SE) as shown in (Appendix 3) to 2,4-D. Close to 100 % percent of the embryos survived under the FETAX controls. The means of LC_{50} of 2,4-D of the three couples in 96-h exposure was 242.09±1.55 mg/l (SE) while the EC_{50} of 2,4-D was 241.37±0.78 (SE) mg/l this was obtained by using probit analysis. There was a high rate of malformation at higher concentration of 200 mg/l and above. This was because of the dose-response relationships. There was 100% mortality in 250 mg/l of 2,4-D in our preliminary studies because there was no hatching of the eggs.

Table 4.5: The 96-h LC_{50} (Mortality); EC_{50} (Malformations) and Teratogenic Index (TI) of 2,4-D on *X. laevis* tadpoles.

Media	LC_{50} 96h (mg/l)	EC_{50} 96-h (mg/l)	TI
2,4-D	242.09 ± 1.55	241.37 ± 0.78	1.003

4.6 Growth effects of 2,4-D and development of the embryos

The results showed that there was a dose related reduction of growth with exposure to 2,4-D (Figure 4.5).

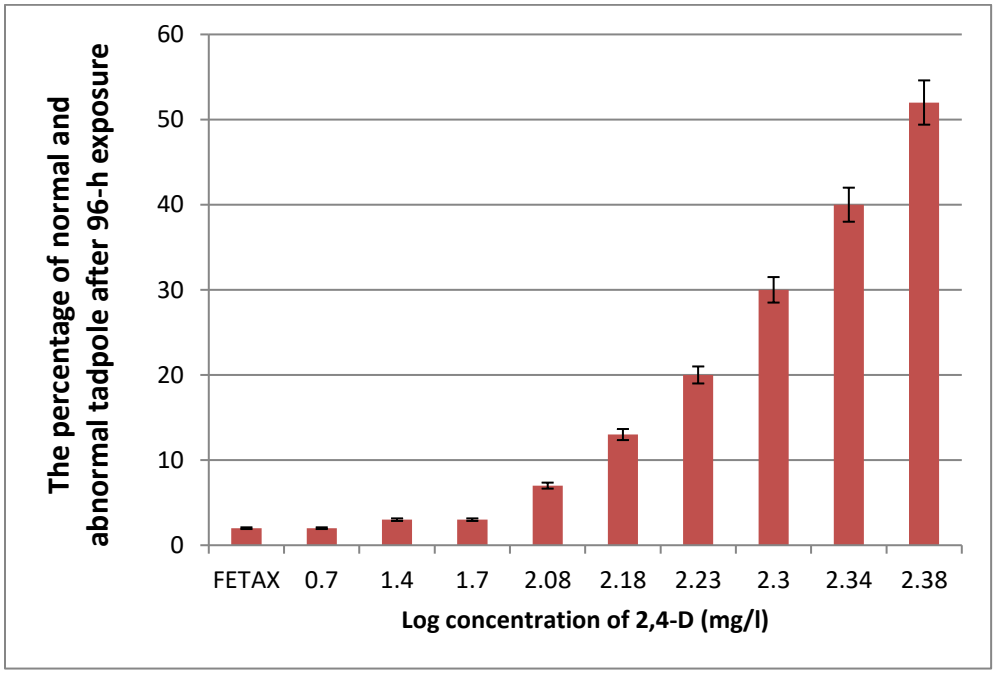
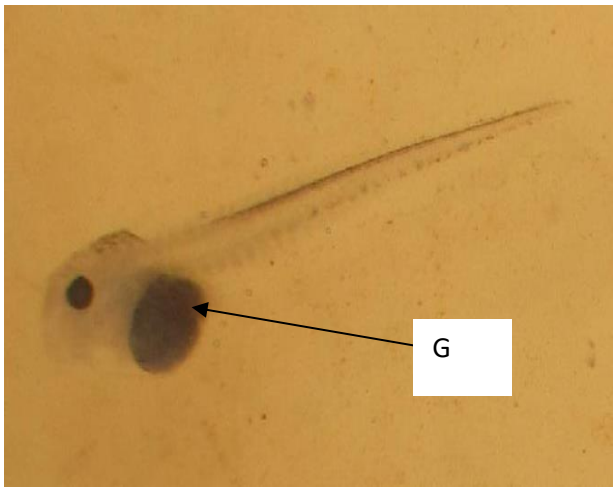


Figure 4.5: The survival and mortality of tadpoles in different concentration of 2,4-D

The *Xenopus* in the control experiments attained stage 46 after 96-h. A stage 46 larva was regonised by the appearence of the hind limb bud, coiling of the gut and the shape of the operculum covering the gills (Plate 4.2). However, the best indicator that the larva had attained stage 46 was the appearance of the hind limb bud and complete coiling of the gut.



Plates 4.2: Normal embryo at 96-h. G:normal gut

Source: Author, 22nd April 2013

After 96-h expoure of the embryos to 2,4-D at different concentrations, the growth of the embryos was measured with micrometer under dissecting microscope. The growth was measured as length from the head to tail. For curved embryos, the measurements were taken as if the embryos were straight, thus following the contours of the embryos. The snout to tail mean size of embryos after 96-h exposure in the control was 6.06±0.05 mm (SE) (n=56),while the mean length of 5 mg/l, 25 mg/l, 50 mg/l, 100 mg/l, 120 mg/l and 150 mg/l of 2,4-D was 6.14±0.07 mm (SE), (n=56), 6.03±0.06 mm (SE), (n=56), 5.84±0.08 mm (SE), (n=55), 5.73±0.13 mm (SE), (n=54), 5.73±0.11 mm (SE), (n=54) and 5.68±0.12 mm (SE), (n=50) respectively (Figure 4.6).

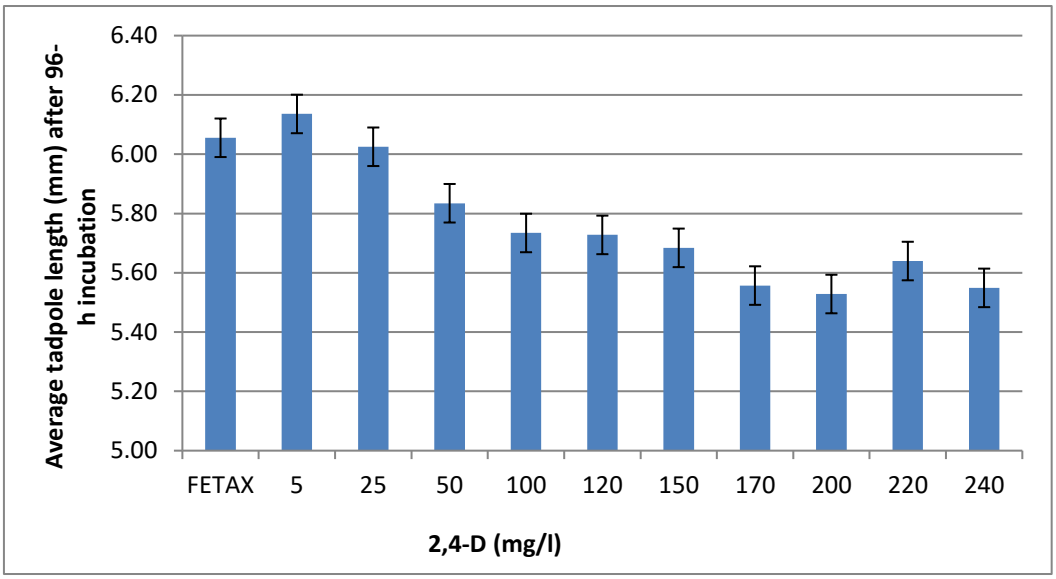


Figure 4.6: Growth responses at different concentrations of 2,4-D

The embryos growth retarded from 170 mg/l, 200 mg/l, 220 mg/l and 240 mg/l with mean length of 5.56±0.10 mm (SE), (n=51), 5.53±0.11 mm (SE), (n=53), 5.64±0.11 mm (SE), (n=48) and 5.55±0.11 mm (SE), (n=49) respectively. This is due to dose-response relationship (the higher the dose the higher the effect).

4.7 Teratogenicity

The transparent nature of the 96-h *Xenopus laevies* tadpole allowed for examination of internal as well as external organs under a dissecting microscope. A tadpole was considered abnormal if it exhibited at least one type of abberration. In deducing the EC_{50} (malformation) Abbort,s adjustment was used to account for the deformities that occurred in the FETAX control (Abbott, 1925) as Y=100×(C-T)/C, where Y=percent malformation,C=percent not malformed in FETAX control,T=percent not malformed in the test media.

An abnormality was scored regardless of its severity. The main types of aberrations observed included flexure of notochord, tail flexure, edema of both head and gut, stunted growth, complete tail curvature and asymmetrical eyes. The FETAX control, 5 mg/l and

25 mg/l had no abnormalities.The minimum concetration that exhibited malformation was 50 mg/l 5.0±4.2% (SE). While the highest exhibited abnormalities was 240 mg/l of 2,4-D 40±4.2% (SE) (Figure 4.7). The deformities increased as the concentration increased as high concentration exhibiting severe malformations at 100 mg/l, 120 mg/l, 150 mg/l, 170 mg/l, 200 mg/l and 220 mg/l with 18.3±4.2% (SE), 20.02±4.2% (SE), 21.7±4.2% (SE), 23.3±4.2% (SE), 26.7±4.2% (SE), and 31.7±4.2% (SE) malformations respectively.

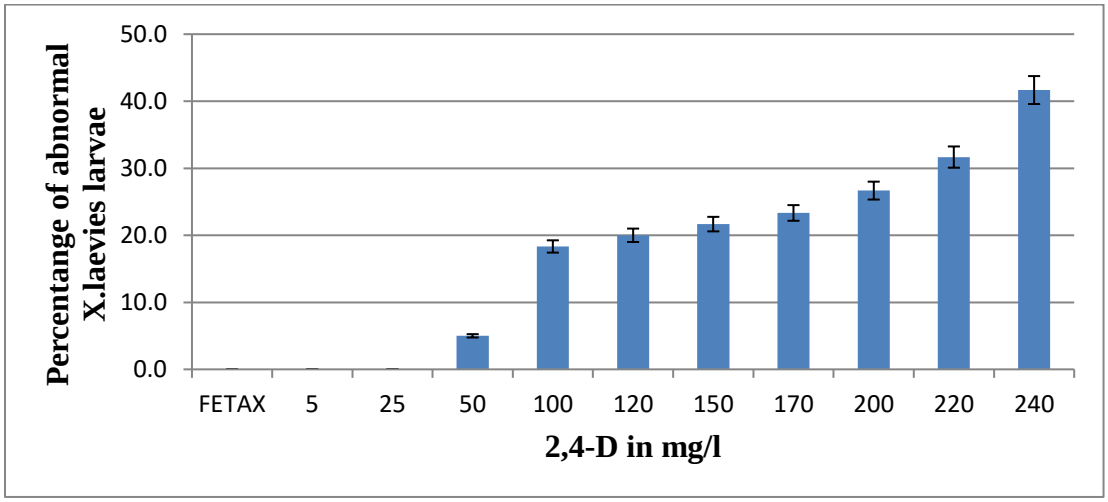


Figure 4.7: Percentage of abnormal X. *laevis* larvae after 96-h exposure to varying concentrations of 2,4-D

2,4-D exhibited different types of deformities; abnormal gut coiling, edema, tail flexure, stunting, assymetrical eyes and flexure of the notocord. There was a higher percentage of stunting deformities with all the concentrations of 2,4-D with 29.5±0.06% (SE) and flexed notocord with the lowest number of deformities 5.4±0.03% (SE). The 50 mg/l and 100 mg/l did not have assymentrical eyes while 120 mg/l, 150 mg/l and 170 mg/l all had 0.9±0.02% (SE) and both 200 mg/l and 220 mg/l had 1.8±0.02% (SE) and 240 mg/l with 0.9±0.02% (SE) giving a total of 7.1±0.02% (SE). There was no obseved malformation at 5 mg/l and 25 mg/l but 50 mg/l and 100 mg/l had mild defeormities. Most of the observed gut malformation include incomplete coiling of the gut and abnormal gut coiling, single loop extruding outside the body was only observed at high concentration of 220 mg/l and 240 mg/l.

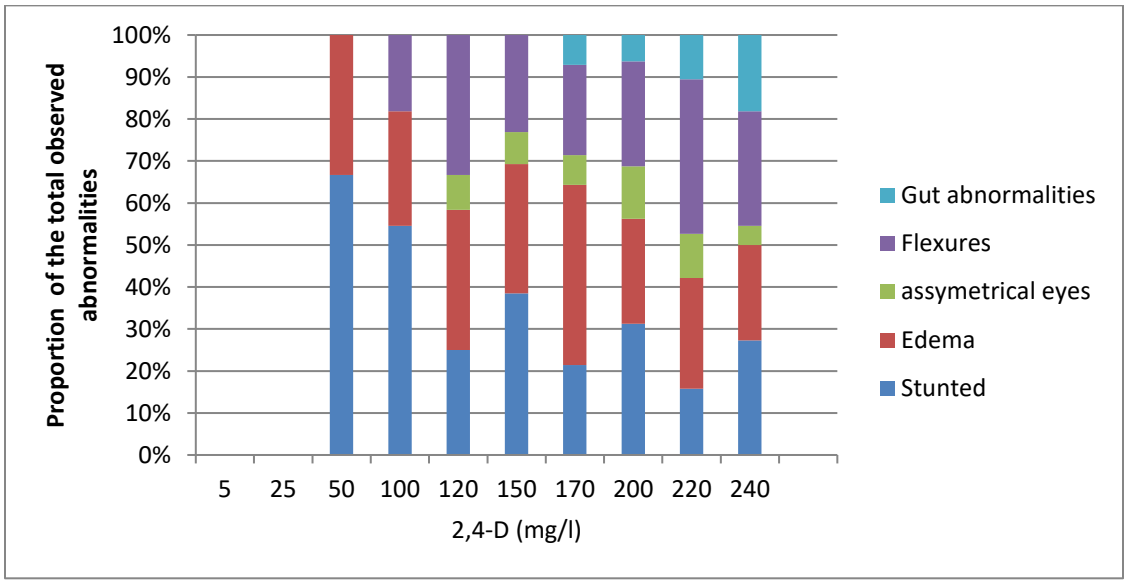
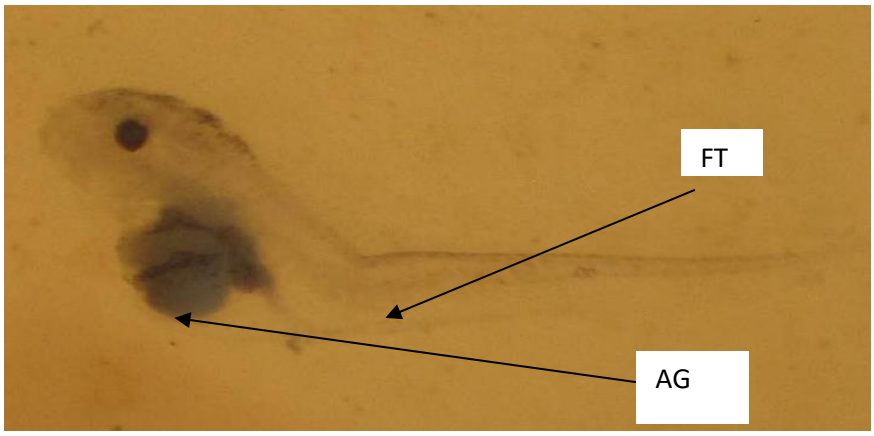


Figure 4.8: The % occurrence of specific abnormalities of tadpoles after 96-h exposure

The gut edemas malformation was observed at low concentration of 50 mg/l 0.6±0.02% (SE) of the total deformities,this included facial edemas. Severe abdominal and cardic edema caused flailure to achieve normal gut development.The notocord flexure were only found at higher concentration of above 200 mg/l. The flexed tails were observed at most of the concentrations meaning it started from lower concentration with a 23.2±0.06% (SE) of the total malformations.

It was found out that most of the abnormal embryos showed multiple malformation for example a tadpole could exhibitwith both gut edema and stunting. Also a tadpole with facial edema with flexed notocord. (Plate 4.3).



Plates 4.3: Multiple malformations in embryo at 240mg/l 2,4-D.FT: Flexed tail,AG:Abnormal gut

Source: Author, 22nd April 2013

The 96-h LC_{50} of 2,4-D in the embryos of *Xenopus laevies* was 242.09±1.55 mg/l (SE) and the EC50 malformation resulted to 241.37±0.78 mg/l (SE). Therefore the teratogenic index (TI) amounted to 1.003, hence 2,4-D is a co-effective teratogen.

4.8 Physico-chemical parameters

The following results are the physicochemical analysis of the water from irrigation canal; the parameters were analyzed in-situ and at the University of Eldoret, Chemistry Laboratory.

Table 4.6: Physicochemical analysis of the sampled water within Ahero Irrigation Scheme

Parameters	Amount	WHO, KEBS drinking water standards
Temperature ^oC	26^0c	-
pH	9.32	6.5-8
EC µS/cm	136	-
Turbidity (NTU)	16.0	$<5^a$ >3 0^b

a- WHO drinking water standards

b- Based on solar disinfection requirement

The electrical conductivity (EC) was found to be 136µs/cm and the water temperature were found to be 26^0C while the pH was 9.32 and turbidity was 16.0 NTU.

4.9 Adsorption of 2.4 D

The experiment was carried out to determine the possibility of removal of pesticide from the contaminated water. The experiment was carried in University of Eldoret chemistry LACB 3, water from Ahero irrigation scheme was passed through Bio-Sand filter to remove the suspended particles before it was spiked with 2,4-D.

4.9.1 Removal of 2,4-D

2,4-D analysis was done in two phases following the procedure stated in 3.9 using HPLC. 2,4-D standard was used to determine the absolute retention time for 2,4-D chromatogram which was used for identification of the target compound in the water sample. The water sample that showed a characteristic "fingerprint" retention time and the shape of the indicator peak were considered as suspected sample with 2,4-D. Figure 4.9 depict a peak for 2,4-D standard when the HPLC conditions were as stated.

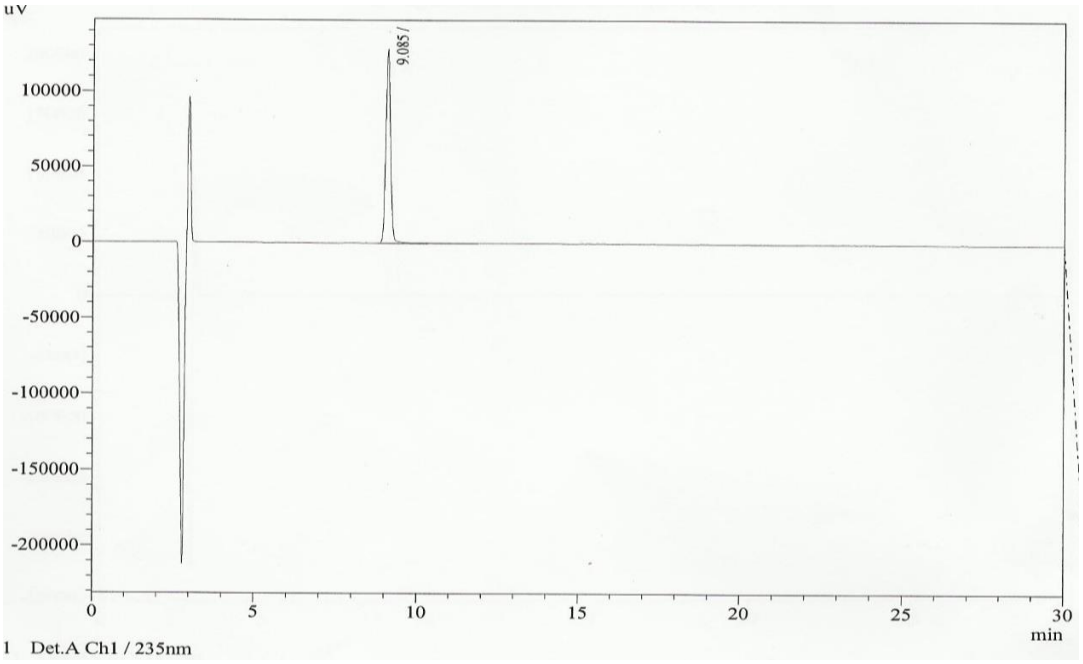


Figure 4.9: High Performance Liquid Chromatogram for 100 mg/l 2,4-D standard

2,4-D standard were analysed to determine the absolute retention time of the peak which was used to confirm 2,4-D. Different concentration of 2,4-D stand were used, these were 10 mg/l, 50 mg, 100 mg/l, 150 mg/l and 200 mg/l which gave elusions or had retention times between 9.085 and 9.087 as shown in the Figure 4.10.

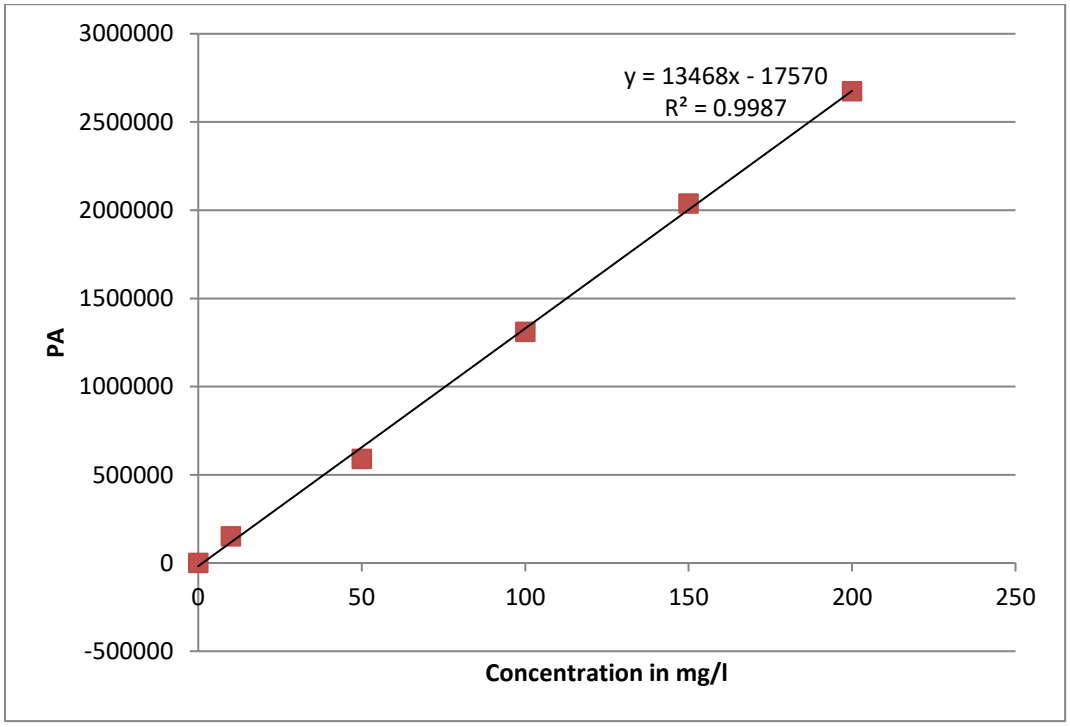


Figure 4.10: The calibration curve for 2,4 –D standard sample

Calibration standard curve is important in determining the 2,4-D that remained in the sample. The peak area of the remaining 2,4-D is used in calculating the sampled that remained.

The percentage of 2,4-D adsorbed was calculated as follows,

$$Adsorption \quad (\%) = \frac{(C_O - C_t)}{C_O} \times 100$$

Where C_o (mg/l) is the initial concentration of 2,4-D, Ct (mg/l) is the residual 2,4-D concentration in the solution at the time (t).

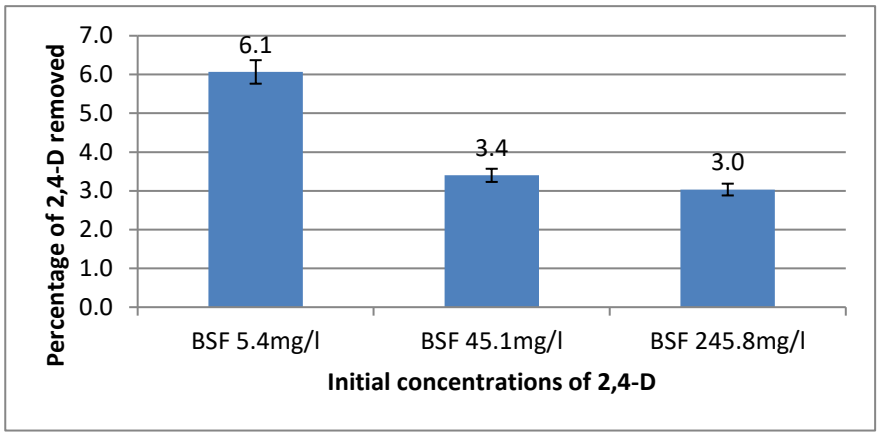


Figure 4.11: The effects of 2,4-D concentration with adsorption at various 2,4-D initial concentrations with Bio-Sand Filter

Bio-Sand filter can remove 99% of the bacterial contaminants (Baumgartner *et al.*, 2007) and (Duke *et al.*, 2006), but has no or less ability to treat other contaminants in drinking water that pose health concerns (Lantagne *et al.*, 2009). The results shows that Bio-Sand filters can remove 2,4-D but in small amounts, with 6.1±0.4% (SE), 3.4±0.1% (SE) and 3.0±0.03% (SE) removal efficiency of the initial concentration of 5.4 mg/l, 45.1 mg/l and 245.8 mg/l respectively.

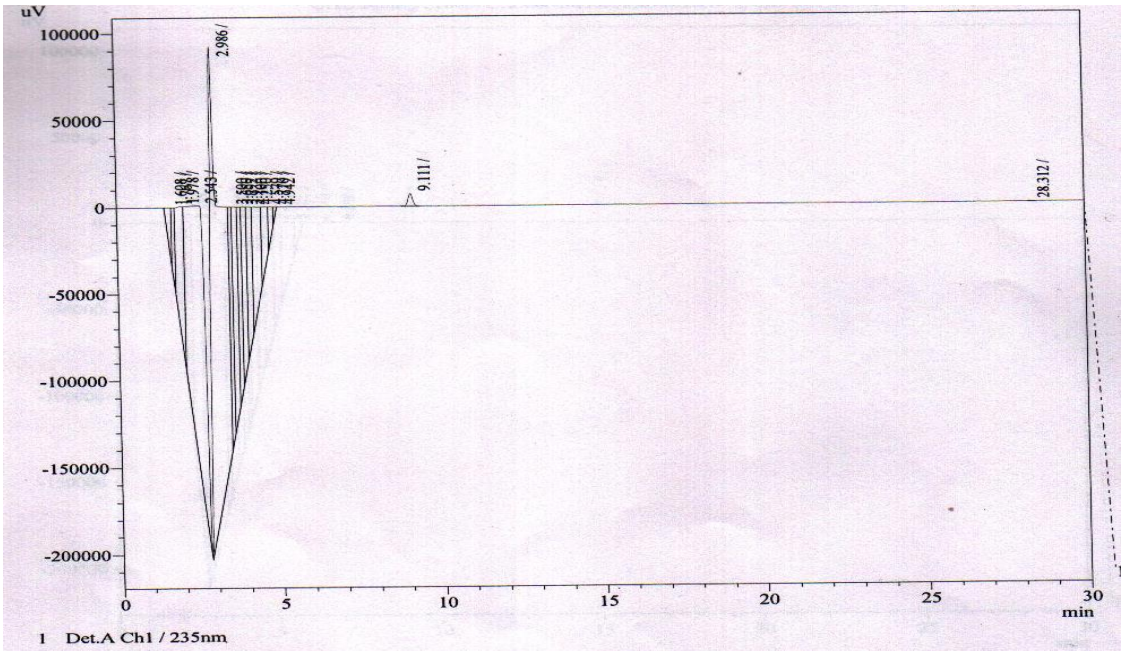


Figure 4.12: HPLC Chromatogram of 5.4mg/l initial concentration of 2,4-D

4.9.2 GAC and Locally Activated Charcoal Bamboo 2,4-D removal

Granular activated carbon (GAC) adsorption is a widely used technology for treating water contaminated with many taste- and odor-causing organics and synthetic organic chemicals (El Diwani *et al.*, 2009) and (Suffet and Wable, 1995). The granular activated carbon (GAC) showed a higher removal of 2,4-D with adsorption capacity of (95.27±0.10%) (SE) compared to the prepared bamboo carbon (91.49±0.15%) (SE) which was obtained after adsorption. This indicated that some of 2,4-D were adsorbed on the activated carbon surface. The uptake of 2,4-D molecules by the adsorbents, and the time required for establishment of equilibrium suggest the effectiveness of the materials for 2,4-D treatment. In order to determine the equilibrium time for maximum 2,4-D uptake, an initial concentration and contact time study was carried out. Effect of initial concentration and time on 2,4-D removal by activated carbon was investigated with varying initial concentrations (5.1, 43.6 and 238.7 mg/L), with 100 grams adsorbent dose (GAC or LACB) , in a fixed bed column with a constant temperature of 26°C and different contact times (30, 60 and 90 minutes). Figure 4.13 shows the adsorption uptake versus the adsorption time at various initial 2,4-D concentrations.

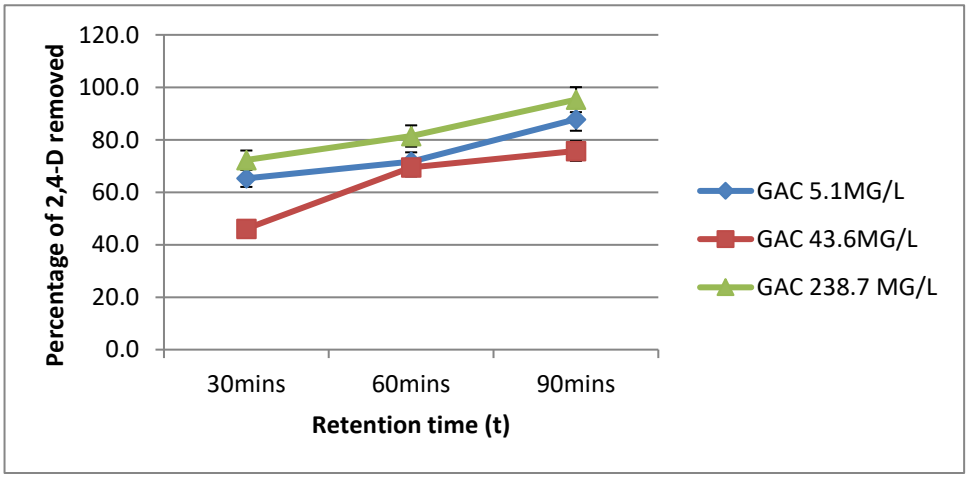


Figure 4.13: Effects of 2,4-D concentration with adsorption at various initial concentration of 2,4-D with Granular Activated Carbon

The removal of 2,4-D by GAC increased with time from 30 minutes to 90 minutes, with the increasing initial concentration from 5.1 to 238.7 mg/L. It is well known that pH of the

solution is a critical factor during adsorption reaction (Budinova and Savova, 2009). In order to determine the adsorption behavior of the 2,4-D, experiments were made in the constant pH 11.5, at a contact time of 30, 60 and 90 minutes with constant 2,4-D concentration (5.1 mg/L) and adsorbent dose (100g).

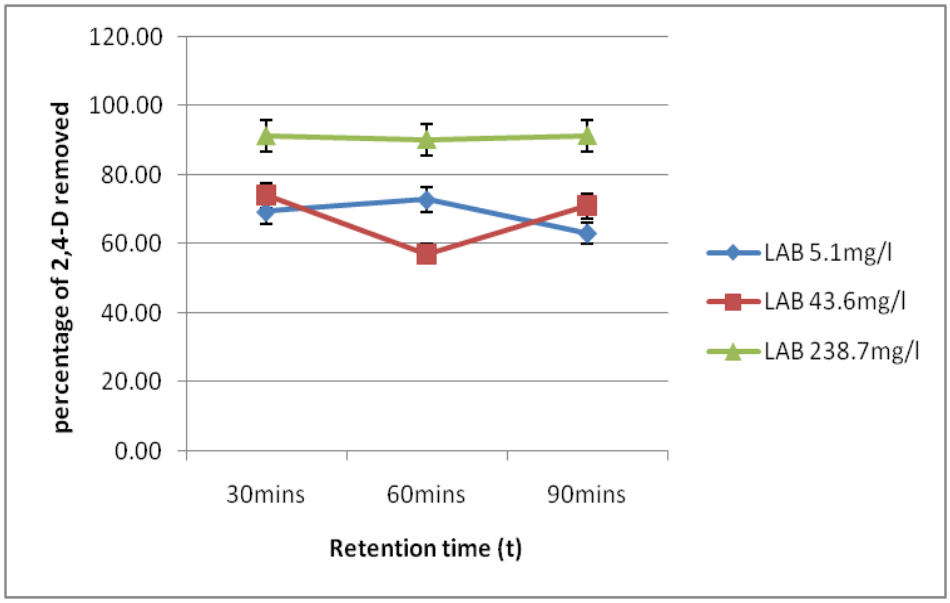


Figure 4.14: The effects of 2,4-D concentration with adsorption at various initial concentration 2,4-D with LACB

Adsorption capacity of bamboo was found to be higher with initial concentration of 238.7 mg/l as shown in Fig. 4.14. There was a significant difference in the removal of 2,4-D by GAC and LACB at initial concentration of 238.7mg/l ($p=0.02$) with the removal efficiencies of (95.27±0.10%) (SE) and (91.4±0.15%) (SE) respectively.

Comparison of the effectiveness of GAC and LACB

In order to determine whether there existed significant differences in the adsorption effectiveness of Granular activated charcoal and bamboo activated charcoal at the retension time of 30, 60 and 90 mins, a t-test was conducted. The results are presented below:

Table 4. 1: T- test results comparing GAC and BAC

	t	**df**	**Significance (2-tailed)**	**Mean Difference**
30	0.24	72	0.081	0.132
60	5.32	72	0.989	0.045
90	1.23	72	0.012	0.523

According to the t-test results above, the p values of activated charcoals30,60, and 90 mins retention time were, were 0.132, 0.045 and 0.523 respectively. All these values were above the 0.05 significance level except for 60 mins retention time indicating that there was a significant difference in at 95% confidence level.

CHAPTER FIVE: DISCUSSION

5.1 Water source

Intensive use of agrochemicals especially herbicides have some adverse effects on surface water and also probably ground water quality in Ahero. As the demand for inexpensive agricultural products continues to increase along with the growing global population, the use of pesticides, herbicides, and fertilizers to increase the productivity of agricultural lands has also increased (Cobb *et al.*, 2012). In the developing world, where regulations are not strictly enforced, impoverished communities near these agricultural activities have suffered effects of pesticide, herbicide, and fertilizer-based contaminants leaching into their water supplies.

Most people from Ahero Irrigation Scheme (AIS) obtain their water from line improved well due to the fact that its clean and reliable water source in the region. This is because of the protection of the well by cementing the entire well at the top and the only way to get water is through pumping. The 22% of the study population of AIS prefer water from the irrigation canal because its more proximal and is less salty. However, it is more turbid, a problem which is most often solved by use of alum coagulant. Some 16% of the study population obtained their water from river Nyando. The Nyando river drain to a vast agricultural area (Bukhalana, 1997), which includes irrigation scheme. The characteristic of the canal water was: High turbidity of 16 NTU and Electrical conductivity of 136 µS/cm and pH of 9.34 (Table 4.6). Chlorination is less effective in alkaline water pH above 8.0 (Lima *et al.*, 2008). Hence chlorination of this water is not effective hence exposing people to micro-organisms.

5.2 Methods of water treatment

The people living around the scheme have different ways of treating water depending on their knowledge and the standard of living. Most people (75%) dropped out of education after only attaining primary school education level and may not have adequate level of knowledge on appropriate way of water treatment.

5.3 Teratogenicity of 2,4 D

The purpose of this study was to determine whether 2,4-D elicit embryotoxicity, developmental and teratogenic effects in the frog embryos. 2,4-D had acute and severe toxicity. The LC_{50} was found to be 242.09±1.55 mg/l (SE) and EC_{50} of 241.37±0.78 mg/l (SE) this was obtained by using probit analysis, this results was in line with the study done by Morgan, (1996) who asses the teratogenic possibility of atrazine and 2,4-d using FETAX and found the 2,4-D EC50 and LC50 values for the buffer were similar at 245 mg/l and 254 mg/l. Teratogenic index of 2,4-D was 1.003, suggesting that 2,4-D is a co-effective teratogenic compound to *X. laevis.* The malformations observed in tadpoles exposed to 2,4-D help demonstrate the specificity of pesticide-induced malformations. 2,4-D can be very toxic, as our preliminary studies showed 100% mortality at 250 mg/l within hours, as the embryos at this concentration did not hatch. Lower concentrations of 2,4-D (50 and 100 mg/L) exposure caused stunting and edemas.

Most edemas in 2,4-D exposed tadpoles were mild and others were severe. These findings concurred with that of Lenkowski *et al.*, (2010) who found that the lower concentrations of 2,4-D (60 and 70 mg/L) exposure caused a significant increase in intestinal malformations and edemas. However, strongly contrast with previous studies showing that malformations occur after exposure to concentrations between 240 and 250 mg/L (Morgan *et al.*, 1996). Vardia *et al.*, (1984) in their study on amphibian showed that 2,4-D is teratogenic to *Xenopus* larvae at lower concentration (LC_{50}=241 mg/l).

The Teratogenic index of 1.003 suggests that 2,4-D is embryolethal, hence it's a teratogen. Growth retardation of the embryos at the end of 96-h suggests that effects of 2,4-D on larvae growth occur early in development. This may cause detrimental effects on the frog population as farmers in AIS use around 144g/l was used per acre. Hence large amount of this pesticide is likely to be found in the environment. Small larvae may also take long to reach succeeding stages of development hence become vulnerable to predation in the river. The observed growth impairment of 2,4-D corroborates other studies on the growth retardation in embryos of tadpole of *X. laevis* and on Indian toad (Vardia *et al.*, 1984).

Previous research has indicated negative effects of 2,4-D exposure on hormone dependent oocyte maturation in *X. laevis* (Stebbins *et al.*, 2004). The other predominant abnormalities observed in this study include edema, facial edemas, tail flexures, shortening of tails, flexure of the notochord, asymmetric eyes and stunting. These finding showed that 2,4-D is a developmental toxicant.

2,4-D have mild and severe teratogenic effects to amphibians as observed in this study; hence the chemical teratogen hypothesis has particular relevance to human health. There is shared long-term exposure with human population through dermal contact during spraying, ingestion via drinking water, bioaccumulation through consumption of contaminated fish and inhalation route as displayed by amphibian.

The effects of 2,4-D was predominantly flexure of the tail and notochord and shortening of the embryos were more apparent at higher concentration of 170 mg/l and above in the assay than the lower concentrations. Park et al., (2010) exposed the aquatic insect *Chironomus riparius* to low concentrations of 2,4-D and observed a statistically significant change in sex ratio of 40% male versus 60% females. In addition, a significantly higher percentage of mouthpart deformities were observed as significantly *C. riparius* exposed to 0.1 μg L−1 of 2,4-D compared to the control group (ibid.). A study by Rodriguez et al., (1994) also showed certain effects of 2,4-D exposure on the reproductive system of crabs (*Chasmagnathus granulate).* Prolonged exposure to 2,4-D has been associated with reduced sperm counts and increased sperm abnormality in both humans and animals (Swan *et al.*, 2003). It has also been strongly linked to extensive and profound developmental neurotoxicity and other fetal growth retardation in Laboratory animals and human cell cultures, suggesting similar effects to humans via exposure during pregnancy or early childhood (Rama *et al.*, 1995).

5.4 Bio-Sand filter, GAC and LACB removal efficiency

This study attempted to remove toxic concentration of 2,4-D from the AIS water. Chemically activated charcoal was produced and applied. Locally activated charcoal from bamboo charcoal was applied in conjunction existent POU treatment options. The effectiveness of the locally activated charcoal from bamboo compared with the commercially available granular activated carbon. Since Bio-Sand filters are the most common treatment option currently used in some parts of Kenya. In retrofitting the existing Bio-Sand filters, the major performance and related design criteria were identified as being critical. The activated carbon granular media or locally activated charcoal from bamboo and traditional sand media would need to work effectively together.

The highest Bio-Sand filter removal for 2,4-D was 6.1±0.4% (SE) at 5.4 mg/l initial concentrations this could be due to adsorption of 2,4-D by the sand to the unfilled spaces between the sand, but this removal is not efficient enough for a large amount pesticide that could be present in water exposing people to risk. High removal efficiencies of 2,4-D was achieved by granular activated carbon and activated charcoal from bamboo with the removal efficiencies of (95.27±0.10%) (SE) and (91.49±0.2%) (SE) respectively from initial concentration of 238.7mg/l of 2,4-D. A study done by Safford and Lackey (2014) on the development of a dual media biological sand filter with added component of activated carbon for use in Vietnam found that activated charcoal was able to remove p-nitrophenol up to 96.8%.The performance criterion could be addressed if activated charcoal were placed in a separate column connected directly to the effluent port of the Bio-Sand filter, then both systems would work independently of each other and the charcoal residue could be removed by filtration with either cloth fabric just before the outlet pipe (Figure 5.1). The option of adding activated charcoal into an existing Bio-Sand filter have several drawbacks; the biologically active layer in the Bio-Sand filter or schmutzdecke, could be affected and also the activated carbon will need to be replaced from time to time depending on the influent concentration of target impurities in the source water. The reported removal efficiency for activated carbon for 2,4-D is 99.0% (Aly and Faust, 1965) and that of wood charcoal with removal efficiency of 92.7% (Alam *et al.*, 2000). Our study presents an

affordable option for removal of both microbiological and chemical contamination of Ahero Irrigation Scheme water (Fig 5.1).

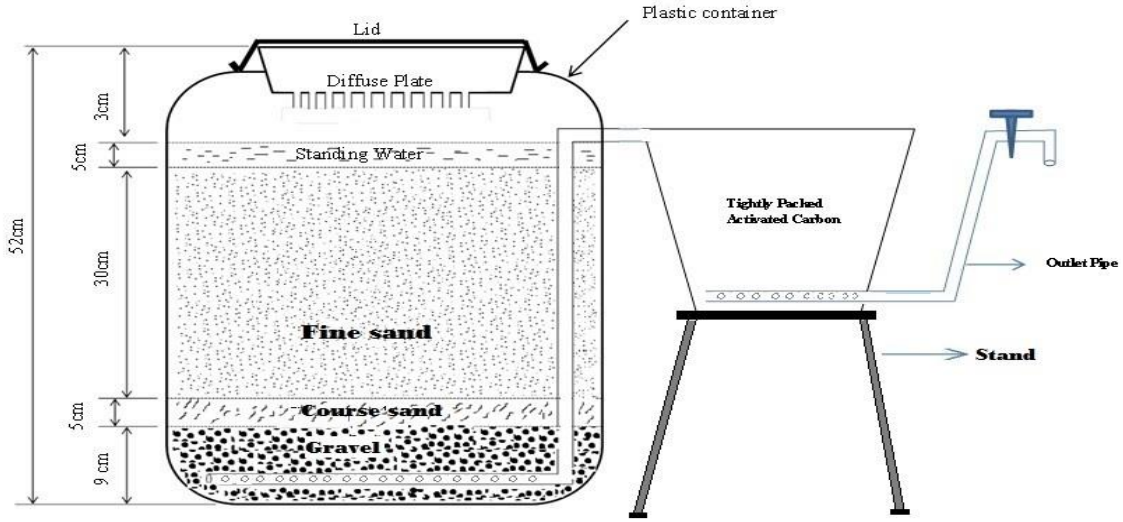


Figure 5.1: The proposed schematic representation of BSF in series with GAC or LACB

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The people living around Ahero irrigation scheme draw their water from lined improved well, Nyando River, irrigation canal, pump borehole and rain water reservoir. The most preferred water source to the villages is line improved well. Due to the saltiness of water drawn from the well and the long distance to the source, some people draw their water from irrigation canals. Close to half population of people around the scheme prefer to us chlorine as a method for water treatment.

2,4-D have shown to cause teratogenicity to the wild Xenopus *laevis* embryos exposed with a teratogenic index of 1.003 which signify that the herbicide is a co-effective teratogen. The predominant malformation that occurred to embryos due to exposure to 2,4-D were stunting, flexed tail, shortening of tails, gut edema, facial edema and flexure of the notochord, Gut malformation was also observed. Flexure of notochord was only seen at a higher concentrations of 2,4-D.

Bio-Sand filter was able to remove pesticide in small amounts at 6%, while Granular Activated carbon removed almost all 2,4-D (95%). Locally activated charcoal from bamboo proved also to be effective in removing 2,4-D with the removal efficiency of 91%. Therefore, production of activated carbon could be adapted to almost any environment by using locally available bamboo and CaCl, an inexpensive and accessible activating chemical. Some studies shows that NaCl which is a table salt can also be used as an activating chemical. Treatment system coupled with the existing water filtration system will act as a dual media filter to provide the greatest degree of treatment.

6.2 Recommendation

- Finding and using substitute chemical for pest and weed control which is easily

degradable and have less environmental and human impacts.

- Carry out water quality analysis to validate the levels of 2,4-D in the irrigation schemes.
- Adaptation of improved Bio-Sand filters as a small-scale purification process. For people who use the raw water from rivers and irrigation canal for drinking and cooking purpose. The improved Bio-Sand filter reduces the amount of organic contaminant such as pesticides, removes harmful micro-organisms that could be present in the water and reduce water turbidity making it clean and safe for consumption.
- Testing for other available wood material as a raw carbon source for activated carbon production should be investigated as bamboo will not be available in all locations.

6.3 Further Research

- Further studies to be done on the epidemiological studies on association between 2,4-D and its degradation products used as an agro chemical and its health-related issues and also the amount that could be found in the environment. These finding can be used by the ministry of health or public health to sensitize community on the safe measures of the use of agro-chemicals in order to alleviate the risks.
- Epidemiology studies should be done in Ahero Irrigation Scheme to determine whether malformation in *X. laevis* frog show a correlation with a suspected human health.
- Further research to be done on the removal of other type of pesticide contaminants where other people practice different types of agriculture.
- Further research on how best to in-cooperate Bio-Sand filter with activated carbon without increasing the complexity of the sand filter.
- Further research to be carried out on the time of use of the locally activated charcoal from bamboo as Granular activated carbon has six-month time of use before replacement as recommended by the manufacturers.

- Further research on the best way of disposal because, after being exhausted, it is likely to create disposal problems and its burning may lead to additional air pollution problems

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APPENDIX 1: Pesticide and water source survey in Ahero rice irrigation schemes
IDENTIFICATION

1. Date: ____/____/2011 2. Name of interviewer: _____

3. Tel No: _____4. Village: _____

RESPONDENT'S CHARACTERISTICS

I. Household head respondent's gender:

	1.Female		2.Male

II. Schools and colleges attended

	1. Primary		2. Secondary
	3. Vocational/Village polytechnic		4. Tertiary College
	5. University		6.Seminar(s)/Workshop(s) or

III. How long have you lived in_____ scheme?

	1. less than 6 months		2. 6 months to 1 year
	3. 1to 2 years		4. More than 2 years
	5. University		6.Seminar(s)/Workshop(s) or shortcourse(s)

SECTION A: WATER SOURCE

I. Where did you get your drinking water today?

	1. Pump borehole		2. Lined (improved) well
	3. River		4. Open well dug in river bed/
	5. Irrigation canal		6. Tap water
	7. Rain water reservoir		

II. Which is the closest water point?

	1. Pump borehole		2. Lined (improved) well
	3. River		4. Open well dug in river bed/
	5. Irrigation canal		6. Tap water
	7. Rain water reservoir		

III. Which is the preferred?

	1. Pump borehole		2. Lined (improved) well
	3. River		4. Open well dug in river bed/
	5. Irrigation canal		6. Tap water
	7. Rain water reservoir		

IV. How long does it take to walk to this source of water?

	1. Less than 15 minutes		2. Between 15 and 30 minutes
	3. More than 30 minutes		

V. Why do you get water from there? (*Multiple responses accepted*)

	1. Water source is closest		2.Water is clean
	3. No waiting		4. Reliable/ usually water
	5. Regular source dry/not working		6. Tap water
	7.Other(*Specify*:_____)		

VI. Who normally fetches water for your household? (*Multiple responses accepted*)

	1. Girl		2.Boy
	3. Woman		4. Man

VII. Did you do anything to the water you collected most recently? (*Multiple responses accepted*)

	1. Washing		2.Cooking
	3. Drinking		

a) What did you do with drinking water you collected?

	1. Nothing		2. Boil
	3. Filter with a cloth		4. Filter with sand/ceramic filter
	5. Chlorinate		6. Alum
	7. Others (specify:		

b) What did you do with the cooking water you collected?

	1. Nothing		2. Boil
	3. Filter with a cloth		4. Filter with sand/ceramic filter
	5. Chlorinate		6. Alum
	7. Others (specify:		

c) What did you do with the washing water you collected?

	1. Nothing		2. Boil
	3. Filter with a cloth		4. Filter with sand/ceramic filter
	5. Chlorinate		6. Alum

	7. Others (specify:		

SECTION B: PESTICIDES

1. List the type of pesticide that you applied in the rice paddy…….
2. Where do you get these pesticides…….
3. When are you applying these pesticide……..
4. What amounts are you applying per acre……...
5. How many time do you apply these pesticide……...
6. How do you decide on the rate of application of these pesticides……
7. Who applies these pesticides…….

APPENDIX 2: Agro-chemical, farmers and NIB stockiest and suppliers' pesticide survey

1. Do you stock herbicides in your shop ……...
2. List the type of pesticide applied in the rice paddy……...
3. Which one is preferred by farmers and why …….
4. Generally what amounts of these pesticides are applied per acre …….
5. Indicate the time of application……..
6. How many times do you apply this pesticides ……..
7. How do you decide on the rate of application of these pesticides……..
8. Explain why this pesticide is preferred……..

APPENDIX 3: Report on the embryo's survival exposed to 2,4-D

	% Survival	% Mortality
N	10.00	10.00
Mean	82.50	17.2000
Std. Error of Mean	5.58	5.67
Std. Deviation	17.66	17.92
Variance	311.83	321.07
Sum	825.00	172.00

Appendix 3 CONT'D: Report of abnormality of exposed embryos

	%Abnormality	%Normality
N	11	11
Mean	17.13	80.90
Std. Error of Mean	4.27	3.38
Std. Deviation	14.16	11.21
Variance	200.36	125.71
Sum	188.40	889.90

APPENDIX 4: Report on the removal of 2,4-D by BSF at several initial

Appendix 3 CONT'D: Report on growth (mm) of embryos exposed to 2,4-D at several concentrations

	FETAX	5 mg/l	25 mg/l	50 mg/l	100 mg/l	120 mg/l	150 mg/l	170 mg/l	200 mg/l	220 mg/l	240 mg/l
N	56	56	56	55	54	54	50	51	53	48	49
Mean	6.06	6.14	6.03	5.84	5.73	5.73	5.68	5.56	5.53	5.64	5.55
Std. Error of Mean	.05	.07	.06	.08	.13	.11	.12	.10	.10	.11	.11
Std. Deviation	.41	.44	.43	.59	.93	.83	.85	.74	.75	.74	.80
Variance	.17	.19	.18	.35	.86	.69	.73	.54	.56	.55	.64
Sum	339.10	343.60	337.40	320.90	309.65	309.30	284.20	283.40	293.00	270.70	271.90

concentrations

	BSF5.4 mg/l	BSF45.1 mg/l	BSF245 mg/l
N	3	3	3
Mean	6.07	3.40	3.03
Std. Error of Mean	.43	.06	.03
Std. Deviation	.75	.10	.06
Variance	.56	.01	.00
Sum	18.20	10.20	9.10

Appendix 4 CONT'D: Report on the removal of 2,4-D by Granular Activated carbon and activated bamboo(BM) at 5.1 mg/l initial concentration.

Initial concentration 5.1 mg/l		N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed
MIN30mins	GAC	3	65.30	2.85	1.64	.34
	BM	3	69.30	5.70	3.29	
MIN60mins	GAC	3	71.63	4.80	2.77	.72
	BM	3	72.77	1.42	.82	
MIN90mins	GAC	3	87.83	5.90	3.40	.26
	BM	3	63.07	2.12	1.23	

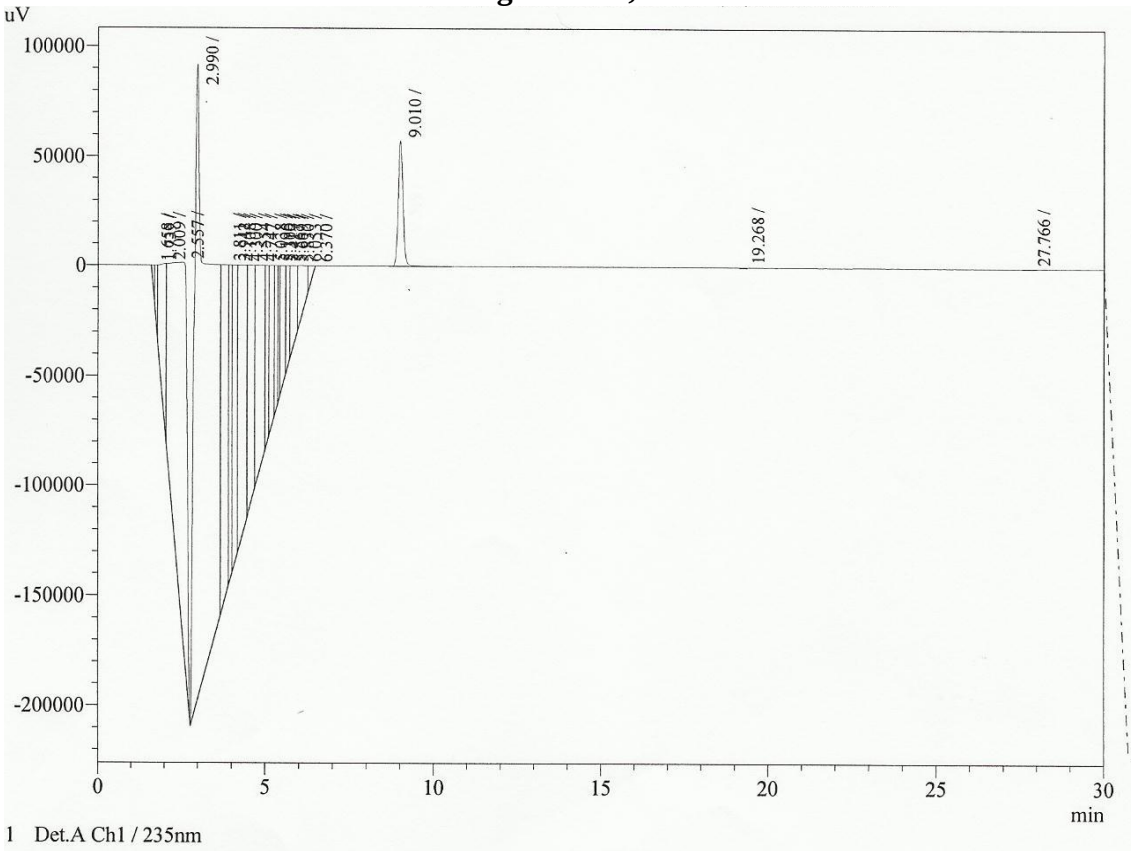
APPENDIX 4 CONT'D: Report on the removal of 2,4-D by Granular Activated carbon and activated bamboo (BM) at 43.6 mg/l initial concentration

Initial concentration 43.6 mg/l		N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
MIN30mins	GAC	3	46.10	26.14	15.09	.15
	BM	3	73.97	6.50	3.76	
MIN60mins	GAC	3	69.57	3.23	1.87	.75
	BM	3	57.03	4.04	2.33	
MIN90mins	GAC	3	75.73	4.22	2.44	.35
	BM	3	70.87	6.80	3.93	

APPENDIX 4 CONT'D: Report on the removal of 2,4-D by Granular Activated carbon and activated bamboo (BM) at 238.7 mg/l initial concentration

Initial concentration 238.7 mg/l		N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
MIN30mins	GAC	3	72.30	2.50	1.44	.60
	BM	3	91.30	1.77	1.02	
MIN60mins	GAC	3	81.43	8.55	4.94	.15
	BM	3	90.20	.78	.45	
MIN90mins	GAC	3	95.27	2.43	0.10	.02
	BM	3	91.49	.26	.15	.

APPENDIX 5: The HPLC Chromatogram of 2,4-D removal



Chromatogram of GAC 5 mg/l contact time 30minutes.